EVALUATION OF THE AMINO ACID METHIONINE FOR BIORATIONAL CONTROL OF SELECTED INSECT PESTS OF ECONOMIC AND MEDICAL IMPORTANCE

By

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Integrated pest management (IPM) strategies were developed in an effort to control pests with fewer pesticides. However, because of the misuse of pesticides and the failure to adopt IPM practices pesticide use is higher than ever. An alternative to conventional broad-spectrum pesticides is the use of biorational compounds; those that pose minimal risk to the environment and are specific to the target pests.

The recent discovery of the CAATCH1 system in the midgut of the tobacco hornworm (THW), *Manduca sexta*, has revealed a novel means to control certain insect pests. This membrane protein works in alkaline conditions as both an amino acid transporter and also independently as a cation channel. However, the amino acid L-methionine blocks amino acid transport thus altering the ion flow.

Bioassays involving the tobacco hornworm, Colorado potato beetle (CPB), *Leptinotarsa decemlineata*, and the yellow fever mosquito (YFM), *Aedes aegypti*, were conducted to determine the insecticidal properties of this compound. L-methionine in artificial and natural diets resulted in the mortality of 50 to 100% in concentrations of 0.3% and higher for THW and CPB. Feeding rates and larval development also were affected with reduced levels (>0.1%) of L-methionine. Bioassay trials involving YFM larvae were similar, concentrations greater than 0.1% L-methionine produced mortality rates of 70 to 100%. All three species responded better to higher concentrations of L-methionine than to *Bacillus thuringiensis*, the most commonly used and commercially available biorational pesticide.

Field trials and non-target tests also were performed to determine L-methionine effectiveness under natural settings and safety to other organisms. Eggplant yield was not reduced by the application of L-methionine, which effectively controlled CPB larvae on the plants. Furthermore, several beneficial insects that were tested (a predator, a herbivore, and a parasitoid) were not affected by the addition of L-methionine to their diets.

Based on these results, L-methionine was found to be effective in controlling selective agriculturally and medically important insect pest species, yet posed little threat to the crop plants applied to or to non-target organisms. The use of L-methionine as a pesticide, its relationship with insects and its possible use in delaying insecticide resistance were also examined.

CHAPTER 1
THE INTEGRATED PEST MANAGEMENT DILEMMA: ARE CONVENTIONAL PESTICIDES THE ONLY ANSWER?

Introduction

Integrated Pest Management (IPM), the sustainable approach to the management of pest species using a combination of biological, chemical and cultural methods to reduce economic, environmental, and public health risk, was a result of economic losses associated with years of overuse of chemical control leading to resistance problems. The use of IPM strategies have certainly decreased pesticide usage and encouraged the use of methods that ensure a safer environment but many feel that it is not enough. After three decades of research efforts in the United States, IPM as it was envisioned in the 1970s was practiced on less than 8% of U.S. crop acreage based on Consumers Union estimates—well short of the national commitment to implement IPM on 75% of the total U.S. acreage by the end of the 1990s (Ehler and Bottrell 2000). This means that farm practices have changed little since the national IPM initiative was established in 1994 to implement biologically based alternatives to pesticides for controlling arthropod pests. It should be noted that the low percentage of IPM practices on commercial U.S. farmland may possibly be related to the lack of sufficient reporting means and actually may be higher than believed when the local growers and homeowners are included. However, the United States is considered the worlds' largest user of chemical pesticides, accounting for nearly 50% of total worldwide production and shows no sign of slowing (Deedat 1994). Pesticides remain the primary tool of pest consultants and farmers, because of the lack of economic incentives to adopt alternative strategies that require more effort to

implement, produce unpredictable results, and require new knowledge (Barfield and Swisher 1994; Ehler and Bottrell 2000).

Importance of IPM in Florida and Surrounding States

Considerable effort has been devoted to developing IPM programs in Florida because of its unique pest problems and crop production systems, sensitivity to chemical pollutants, and increased urbanization (Capinera *et al.* 1994; Rosen *et al.* 1996). The necessity for developing IPM protocols for Florida's major plant and animal pests was underscored in a new statewide initiative. In November 1999, the Institute of Food and Agricultural Sciences (IFAS) at the University of Florida launched Putting Florida FIRST —**F**ocusing **I**FAS **R**esources on **S**olutions for **T**omorrow (Florida FIRST 1999). The Florida FIRST initiative was created (with input from stakeholders) to define the role of IFAS in shaping Florida's future in the 21st century. Increasing concerns (expressed repeatedly by Florida's scientific community and the general public) about environmental contamination, food safety issues, and human and animal health problems resulting from the indiscriminate use (and often misuse) of pesticides are making existing methods for pest management obsolete. Successful implementation of "true" IPM, as it was envisioned by those who envisioned the original concept, will have the added benefit of helping Florida ". . . enhance natural resources, provide consumers with a wide variety of safe and affordable foods, . . . provide enhanced environments for homes, work places and vacations, maintain a sustainable food and fiber system, and improve the quality of life. . ." (Florida FIRST 1999).

This effort to promote IPM programs in the state of Florida also benefits the surrounding states. For example, solanaceous crops produced in the southeastern U.S. (such as tomato, tobacco, eggplant, peppers and potato) are subjected to the same

defoliation and fruit damage from various lepidopteran and coleopteran pests that also threaten Florida. The tomato pinworm [*Keiferia lycopersicella* (Walshingham) (Lepidoptera: Gelechidae)], armyworms [*Spodoptera* spp. (Lepidoptera: Noctuidae)], the Colorado potato beetle [*Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae)], and hornworms [*Manduca* spp. (Lepidoptera: Sphingidae)] are some examples of pests that threaten both conventional producers and homeowners alike. For example, the estimated loss from and the cost of control of the tobacco hornworm, the number-one pest in tobacco crops in Georgia, reached $1.5 (and $2.3 million), respectively, for the years 1996-1997 (Jones and McPherson 1997). From 1992-1998, tomato, eggplant, and pepper producing areas in the Southeast had a total of 1,247,000 pounds of endosulfan applied over 270,000 acres (Aerts and Neshiem 1999; Neshiem and Vulinec 2001). The cost of insecticides applied in Florida tomato production alone for 1993-1994 amounted to approximately $1,052/hectare for a total of $2.1 million; and rose to $2550/acre, totaling $103M for the 1996-1997 season (Aerts and Neshiem 1999; Schuster *et al.* 1996). The use of pesticides in Florida tomato production is high because tomatoes account for 30% of the total vegetable-crop value and 13% of the total vegetable acreage for the state, with 99% of production aimed toward the fresh market (Schuster *et al.* 1996). For Florida potato producers, the cost of applying pesticides from 1995-1996 was $11.5M, and 96% of total Florida eggplant-crop acreage was treated with chemical insecticides (mainly methomyl and endosulfan) (Neshiem and Vulinec 2001). In addition to the monetary cost of pesticide use, commonly used insecticides such as endosulfan and fenvalerate show a high degree of toxicity to parasitoids of the tomato pinworm, thus negating the benefits of predation by natural enemies (Schuster *et al.* 1996). These figures may be the result of the "more is better" attitude of producers who want to avoid

all risk of insect damage by using more applications and stronger pesticides (Schuster *et al.* 1996).

Problems Associated with Pesticide Misuse

The use of pesticides is not completely ruled out under IPM strategies, but rather IPM encourages responsible use to minimize environmental harm and to protect human safety and health (Deedat, 1994). However, the misuse (both intentional, in terms of "more is better;" and unintentional, as in agricultural runoff) also has resulted in resistance in some of the target pests. For example, surveys in North Carolina have shown that the Colorado potato beetle has become resistance to fenvalerate, carbofuran, and azinphosmethyl as a result of control failures in the field (Heim *et al.* 1990). Resistance to insecticides has also been observed in more than 450 arthropod pests (Romoser and Stoffolano 1998). Bills *et al.* (2004) found a 38% increase in the number of registered compounds used as pesticides from 1989-2000, and also a 16% increase in pesticide resistance of arthropod species worldwide.

Losses are not limited to agricultural systems alone. Across Africa for example, populations of insecticide-resistant mosquitoes are the result of a variety of mechanisms, including exposure to pesticide residues in agricultural runoff, mutation of target sites, and migration of resistant populations into areas where there were no previous problem (FIC-NIH 2003). Parts of southwest Asia have seen a resurgence of malaria in some areas where it was considered eradicated (due to a combination of resistance and the economics associated with control of mosquito vectors) (Deedat 1994). The importance of this example becomes even more relevant when one considers that one million individuals die every year as a result of malaria, with upwards of 500 million cases per year (Centers for Disease Control 2003). The existence of other mosquito-borne diseases

such as Dengue fever, yellow fever, and West Nile virus to name just a few, put countless millions more at risk. It would be dangerous to think that these diseases only occur in underdeveloped countries and not the United States. Integrated Pest Management practices also should be adopted for controlling the medical and veterinarian important insect vectors of these and other diseases.

Biorational Compounds: An Alternative to Traditional Chemical Insecticides

One way to reduce this reliance on traditional chemical pesticides and delay resistance is by increasing the variety and use biorational compounds. Biorational compounds are effective against selected pest species but are innocuous to nontarget or beneficial organisms; and have limited affect (if any) on biological control agents (Stansly *et al.* 1996). Biorational compounds include detergents, oils, pheromones, botanical products, microbes, and systemic and insect growth regulators (Perfect 1992; Wienzierl *et al.* 1998). Their safety lies in the low toxicity of the compound to nontarget organisms and the compound's short residual activity in the field. For example, *Bacillus thuringiensis isrealensis* (*Bti*) currently is one of the most widely used microbial pesticides for controlling aquatic dipteran pests (*i.e.*, mosquitoes and black flies) because of its selectivity to this group and minimal nontarget effects (Glare and O'Callaghan 1998). However, resistance to *Bt* products has occurred in many species of lepidoptera from overuse of *Bacillus thuringiensis kurstaki*, and in some mosquito species to *Bti,* thus showing the need for alternatives to these compounds that are still effective (Brogdon and McAllister 1998; Marrone and Macintosh 1993). In addition to resistance, other problems are associated with the use of microbial control agents. Cook *et al.* (1996) discussed potential hazards, not properly identified in the planning stages, of displacement of native microorganisms, allergic responses in susceptible humans and

animals, and eventual toxicity to nontarget organisms. Because of these problems, alternatives are needed to prevent another crisis like the one from which IPM originally arose.

CHAPTER 2
HISTORY OF THE USE OF AMINO ACIDS AS A MEANS TO CONTROL INSECT PESTS

Non-Protein Amino Acids

One avenue of pest management explored in the field of biorational pesticides is the use nonprotein amino acids. Secondary plant materials such as these serve many functions in insect-plant relationships from attractants and repellents to crude insecticides (Dahlman 1980). Only a few nonprotein amino acids have been examined as a potential means to control insect pests. L-canavanine and its by-product of detoxification, L-canaline, have been studied extensively, with a variety of effects ranging from developmental deformities to aberrant adult behavior (Dahlman and Rosenthal 1975; 1976; Rosenthal *et al.* 1995). L-canavanine is found mainly in leguminous plants, including several economic species (Bell 1978; Felton and Dahlman 1984). It is believed that plants produce this allelochemical for protection against feeding by phytophagous insects and herbivores (Rosenthal 1977). The mode of action for canavanine can be traced to several metabolic processes, including disruption of DNA/RNA and protein synthesis, arginine metabolism, uptake, anomalous canavanyl protein formation, and the reduction of active transport of K^+ in the midgut epithelium (Kammer *et al.* 1978; Racioppi and Dahlman 1980; Rosenthal 1977; Rosenthal *et al.* 1977; Rosenthal and Dahlman 1991). In contrast, canaline possesses neurotoxic characteristics with an unknown mode of action (Kammer *et al.* 1978). The species of choice for studies involving nonprotein amino acids has been the tobacco hornworm (THW), *Manduca sexta* (L.) (Lepidoptera: Sphingidae).

L-canavanine exhibits a range of insecticidal effects in artificial diets when exposed to the THW. Dahlman (1977) demonstrated a reduction in consumption of artificial diet containing less than 1% canavanine (w/v) which resulted in a lower body mass and increased developmental time to the adult stage. Fecundity and fertility also was affected by L-canavanine. Rosenthal and Dahlman (1975) showed that concentrations as low as 0.5 mM L-canavanine in the diets of the THW resulted in the reduction of ovarial mass of adults, while Palumbo and Dahlman (1978) showed that concentrations of L-canavanine in agar-based diets resulted in the reduction of chorionated oocyte production in concentrations between 1.0 and 2.0 mM.

Under natural conditions, L-canavanine was found to retard development, and increased the susceptibility of exposed larvae to biotic and abiotic mortality factors (Dahlman 1980). However, field applications of L-canavanine were shown to be impractical because of the expense involved in synthesizing L-canavanine from its source, the jack bean (*Canavalia ensiformis* (L.) DC. (Family: Fabaceae)).

Other sources of L-canavanine (i.e., analogues and homologues) were sought in an attempt to find a more practical source of the amino acid. Structural homologues of canavanine were examined and found to contribute to pupal deformities (and to a lesser degree, to mortality) (Rosenthal *et al.* 1998). Long-chain esters of L-canavanine were found to be more toxic than the parent compound when injected or added to an artificial diet exposed to last instar of THW specimens (Rosenthal *et al.* 1998). Adding amino acids other than arginine (the parent compound to L-canavanine) to diets containing L-canavanine increased deformities and mortality of THW larvae and was attributed to the structure and position of the functional groups on the added compounds (Dahlman and Rosenthal 1982). Although the THW has an effective means of degrading aberrant

proteins (produced by the assimilation of L-canavanine) into newly synthesized proteins; the proteases involved do not efficiently degrade enough to prevent some damage from occurring in the insect (Rosenthal and Dahlman 1986; 1988).

Surprisingly, L-canavanine also was shown to increase the effectiveness of *Bacillus thuringiensis in vivo* by altering membrane properties, mainly gut permeability, and active transport in the midgut of the THW (Felton and Dahlman 1984). However, despite the possible synergistic relationship between the relatively safe *Bt* product and this amino acid, no further research has been conducted to evaluate the combination for future commercial use.

Other species of insects have also been tested for susceptibility to canavanine with a variety of results. Larvae of *Drosophilia melanogaster* Meigen (Diptera: Drosophilidae) showed no deleterious response to lower concentrations of canavanine, but showed mortality increased at concentrations over 1,000 ppm (Harrison and Holiday 1967). Lower concentrations also were ineffective against adult *Pseudosarcophaga affinis* (Fallen) (Diptera: Calliphoridae), with no effect on oocyte development (Hegdekar 1970). Dahlman *et al.* (1979) examined four species of muscoid flies and found greater than 70% mortality at the higher concentration (800 ppm) and decreased pupal weights as concentrations of canavanine increased.

Despite the toxicity of canavanine to some insects, others have evolved detoxifying mechanisms to deal with high concentrations of this compound. Rosenthal *et al.* (1978) attributed the detoxification of canavanine in the bruchid *Caryedes brasiliensis* Thunberg (Coleoptera: Bruchidae) to the beetle's ability to convert canavanine to canaline, another toxic amino acid. Canaline is metabolized through reductive deamination to homoserine and ammonia, with the overall result being the detoxification

of the two antimetabolites. This process actually increases the nitrogen intake from the foodstuff (from the increase of ammonia) (Rosenthal *et al.* 1976; Rosenthal *et al.* 1977). Another insect, the tobacco budworm (*Heliothis virescens* (Fab.) (Lepidoptera: Noctuidae)) was able to metabolize far more canavanine then the bruchid beetle larva ever takes in during its development, suggesting that generalists may have more than a single detoxification mechanism for compounds they may encounter (Berge *et al.* 1986). Metabolism of L-canavanine by the tobacco budworm was attributed to the gut enzyme canavanine hydrolase, and may have been the result of feeding on canavanine-containing plants of the Fabaceae (Melangeli *et al.* 1997).

Essential Amino Acids

In despite of the extensive toxicological research conducted on nonprotein amino acids, another group of amino acids, the essential ones, has been overlooked. One reason this avenue for research has not been pursued is that we do not want to give pests convenient access to an integral part of their diet. The fear of creating a "super" insect (that has been provided with compounds that actually aid in its development) is a rational one. Mittler (1967a; 1967b) found an increase in gustation in *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), with amino acid levels as low as 0.2% concentration in a sucrose solution. Likewise, Sugarman and Jakinovich (1986) found increased gustatory response to both D-and L-methionine by *Periplaneta americana* (L) (Blattodea: Blattidae) adults. Another reason that essential amino acids have not been examined for use as a pesticide is the knowledge regarding the limited mode of action these compounds could be involved with (*i.e.*, an active site or systemic response). Recent studies on the membrane proteins of insects show the possibility of a biophysiological system that can be exploited for insect control with certain essential amino acids.

The Cation-Anion Modulated Amino Acid Transporter With Channel Properties (CAATCH1) System

Cation-Anion modulated Aminoacid Transporter with Channel properties (CAATCH1) is a recently cloned insect-membrane protein isolated from larval midgut/hindgut nutritive absorptive epithelium. This membrane protein exhibits a unique polypeptide and nucleotide sequence related to, but different from, mammalian Na^+-, Cl^--coupled neurotransmitter transporters (Feldman *et al.* 2000). Using a unique PCR-based strategy, the gene encoding CAATCH1 was cloned from the digestive midgut of THW larvae. The unique biochemical, physiological, and molecular properties of CAATCH1 indicate that it is a multifunction protein that mediates thermodynamically uncoupled amino acid uptake, functions as an amino acid-modulated gated alkali cation channel, and is likely a key protein in electrolyte and organic-solute homeostasis of pest insects (Quick and Stevens 2001). In the presence of no amino acids, the cations K^+ and Na^+ are transported through the membrane *via* the channel (Figure 2A). When exposed to proline, the amino acid is transported through the membrane with an increase in cation flow, especially Na^+ (Figure 2B). However, when exposed to methionine, the amino acid transport is stopped and cation flow is altered, mainly the increased flow of K^+ and the decreased flow of Na^+ (Figure 2C). The CAATCH1 system works in alkaline conditions, at a pH optimum ~ 9.5. This alkaline condition is found in the midgut of several species (Nation 2001) and has been attributed to a variety of causes, from the detoxification of plant allelochemicals to amino acid uptake (Giordana *et al.*, 2002; Leonardi *et al.* 2001).

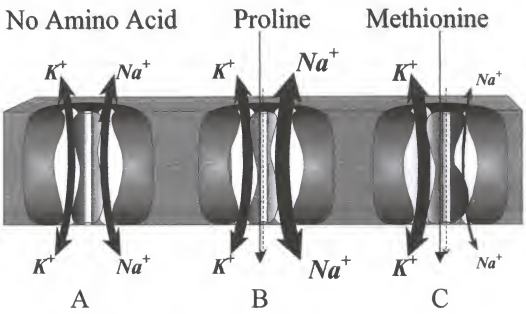

Figure 2. The CAATCH1 system identified from the midgut of the tobacco hornworm (modified from Quick and Stevens 2001). In the presence of no amino acids, ion flow is similar for both K^+ and Na^+ (A). With the addition of an amino acid, flows are changed. When proline is added (B), the transport occurs but the binding of the amino acid increases the ion flow, notably Na^+. However, when methionine is added (C) transport occurs and the binding of the amino acid greatly decreases the flow of Na^+ while K^+ is increased

Several amino acids were found to initiate the blocking action of ion flow through the CAATCH1 protein, including threonine, leucine, and methionine, with the latter producing the greater response, based on CAATCH1 research (Feldman *et al.* 2000; Stevens *et al.* 2002; Quick and Stevens 2001).

Methionine

The amino acid methionine is considered essential in the diets of many organisms. Methionine is considered an indispensable amino acid in humans. Because the body does not synthesize it, uptake of methionine must occur in the diet. The recommended daily allowance of methionine for a healthy lifestyle ranges from 13 to 27 mg/kg/day for infants to full-grown adults (Young and El-Khoury 1996). This amino acid is linked to a decrease in histamine levels, increased brain function, and is found in a variety of sources; with the highest concentration in various seeds, greens, beef, eggs, chicken, and fish (Dietary Supplement Information Bureau 2000). Recently, research has centered on the genetic modification of crop plants to overproduce methionine to increase its nutritional quality (Zeh *et al.* 2001). Wadsworth (1995) discussed using methionine as a feed supplement, as an aid in the therapy of ketosis in livestock, and as a treatment for urinary infections in domestic pets. Onifade *et al.* (2001) examined the use of housefly larvae as protein foodstuffs, and found an increase in body weight gain and erythrocyte counts in rats whose diets were supplemented with fly larvae and methionine. Likewise, Koo *et al.* (1980) suggested dry face fly pupae could be used as a dietary supplement and foodstuff extender for poultry because of the high concentration of methionine. The environmental safety of methionine is well known, as it poses no risk to vertebrates due to a rather high oral LD_{50} of $36g/kg^{-1}$ observed in rats (Mallinckrodt Baker 2001) and also

in its use as a feed supplement for livestock under the trade name of Alimet® (Novus, Inc., St. Louis, MO).

In addition to vertebrates, methionine also is considered an essential amino acid for insects (Nation 2001). Based on research on nutritional requirements for insects, the amount of methionine needed in a diet for survival ranged from as little as 0.0007 mg/mL (for *Aedes aegypti* (L.) (Diptera: Culicidae) to as high as 100 mg/mL (for *Heliothis zea* (Broddie) (Lepidoptera: Noctuidae)) (Dadd and Krieger 1968; Eymann and Friend 1985; Friend *et al.* 1957; Kaldy and Harper 1979; Kasting *et al.* 1962; Koyama 1985; Koyama and Mitsuhashi 1975; Rock and Hodgson 1971; Singh and Brown 1957). Methionine occurs naturally as the L-isomer while the D-isomer (an optical enantiomer) is toxic to many insects and is not found in nature (Anand and Anand 1990). A few exceptions are known, (mainly Diptera and Lepidoptera) that actually are capable of metabolizing the normally unusable D-isomer (Dimond *et al.* 1958; Geer 1966; Rock 1971; Rock *et al.* 1973; Rock *et al.* 1975). The requirement for small amounts of this amino acid (as compared to other amino acids) may be a result of the ability for some insects to synthesize methionine from cysteine (a common sulfur containing amino acid) thus reducing the need to take in exogenous sources of methionine. Jaffe and Chrin (1979) found that *A. aegypti* adults were able to synthesize methionine from homocysteine with the aid of a methionine synthetase. They found this enzyme similar to those common in other metazoans, and found that the levels of methionine synthetase increased with the presence of filarial parasites. They hypothesized that this increase in methionine synthetase was a result of the parasite depleting the host of methionine.

Fertility and fecundity also have been associated with methionine in some insects (mainly *D. melanogaster*,) with the possibility if it being a limiting factor during egg

production (Sang and King 1961). Lack of methionine in the diet of the female may also explain the transfer of methionine in the ejaculate of the male during fertilization (Bownes and Partridge 1987). Methionine plays another role in insect biochemistry, especially in juvenile hormone biosynthesis, inhibitory allatostatins, and storage proteins known as hexamerins. Audsley *et al.* (1999) found that *in vitro* rates of juvenile hormone synthesis in females of the tomato moth (*Mamestra oleracea* (L.) (Lepidoptera: Noctuidae)) were dependent on the concentration of methionine present in the incubation medium. Tobe and Clarke (1985) found a direct relationship between methionine concentration and juvenile hormone biosynthesis in the cockroach, *Diploptera punctata* (Eschscholtz) (Blattodea: Blaberidae), further supporting the idea that methionine plays an important role in insect biochemistry.

Storage proteins, or hexamerins, act as a storehouse for amino acids that can be sequestered for later use in the developmental cycle (Pan and Telfer 1996). Many Lepidoptera have been identified with hexamerins containing high concentrations of methionine and are metabolized during the last larval stage, and presumably used for egg production (Wheeler *et al.* 2000).

Methionine as a potential pesticide has not been overlooked entirely. Tzeng (1988) tested a methionine and riboflavin mixture and found it successful in controlling various pests, including the larvae of *Culex* spp. (Diptera: Culicidae). The mode of action for this mixture was attributed to a photodynamic reaction and the production of oxygen rich radicals (Tzeng *et al.* 1990). Their research led to the use of this methionine compound as a control agent for sooty mold of strawberry (Tzeng and Devay 1989; Tzeng *et al.* 1990) but not as an insecticide.

Discovery of novel means for controlling various insect pests is one tenant of IPM. The amino acid methionine, an environmentally safe organic compound, appears to be a candidate for further study. Before it can be considered for use in controlling insects pests, several issues must be addressed, including the determination of concentrations needed to provide effective control, compatibility with current application systems, safety to nontarget organisms (*i.e.*, beneficial or biological-control agents), and to phytotoxicity.

Research Objectives

Our overall goal was to evaluate the effects of L-methionine, and its amino acid analogues, on the CAATCH1 system putatively in the midgut/hindgut as a means to control different insect pests. The working hypothesis is that the L-methionine only affects the CAATCH1 system and no other system, especially those involving Na+ channels or pumps (*i.e.*, nervous tissue). The L-isomer of methionine was chosen because of the inability of most insect species to utilize the D-isomer. Ideal targets for this research are those pests that cause severe damage to agricultural systems and to human health. Specific objectives were to

- Examine the effects of L-methionine as an insecticide on the larvae of *M. sexta* (Tobacco hornworm), *L. decemlineata* (Colorado potato beetle) and *A. aegypti* (Yellow-fever mosquito) under various conditions
- Determine any adverse effects of L-methionine on plant health to ensure its safe use in a cropping system
- Examine the effects of L-methionine on various nontarget insect species to ensure the environmental safety of L-methionine and thus its compatibility with natural enemies in the context of IPM.

CHAPTER 3
EFFECTS OF L-METHIONINE ON SURVIVAL AND DEVELOPMENT OF THE TOBACCO HORNWORM, *Manduca sexta*, UNDER LABORATORY CONDITIONS

Introduction

Manduca sexta (L.) (Lepidoptera: Sphingidae), the tobacco hornworm (THW), is a widespread species considered an economic pest throughout North and South America. The caterpillar is known for its voracious appetite. In Georgia, the THW was responsible for between approximately $1.2 to $1.5 million in losses and costs for control annually in tobacco from 1997 to 2001 (Jones and McPherson 1997; McPherson and Jones 2002). In addition to its well-earned reputation as an agricultural pest of solanaceous crops, the THW has shown to be resistant to common pesticides (such as endrin and endosulfan), with the possibility of cross-resistance (Bills *et al.* 2004).

The THW also is very important to scientific research outside the arena of economic entomology, with studies ranging from molecular-based research to ecological and physiological research, mainly because of its availability and ease in culturing (Dwyer 1999). One research area of interest to scientists involves the chemistry and physiology of the midgut. Insect control (or the development of new insecticides) was probably not the main purpose of the research that resulted in identifying the CAATCH1 protein, yet it became the basis of our research project.

Because little information is available on the insecticidal properties of methionine, several baseline experiments were necessary to determine that concentrations of this amino acid to test. It also was necessary to test L-methionine and THW

interaction in a variety of ways, including artificial diet, natural diet (excised leaves, whole plant, and choice tests. The purpose of this portion of this study was to determine whether L-methionine was detrimental to the survival and development of the THW and to determine if L-methionine could be used to control this species.

Materials and Methods

Eggs of THW were obtained from the insectary of North Carolina State University, and were held in 26.4L x 19.2W x 9.5H (cm) clear plastic rearing chambers with a hardware cloth (to facilitate cleaning) (Figure 3-1). Florida Reach-In Units (FRIUs) were used to control the environment for the rearing containers (Walker *et al.* 1993) Containers were held at 27° C, 60% relative humidity, and a 16L:8D photoperiod in FRIUs with either artificial or natural diet (excised eggplant leaves or whole plants) depending on the pending experiment. Neonates were allowed to feed for 2 days after eclosion before being transferred to treatment groups. A camel hair brush was used for transferring larvae, to minimize the risk of damage.

Diets and Survivorship

The artificial diet was prepared using the procedures outlined in Baumhover *et al.* (1977) with the inclusion of L-methionine for the treatment concentrations of 0.1%, 0.3%, 0.5%, 1.0%, 3.0%, 5.0% and 10.0% (wt/wt). The artificial diet was changed on a regular basis to prevent desiccation and fungal growth. Larvae were exposed to the artificial diet in the clear plastic rearing chambers with a hardware cloth, and kept in the FRIUs programmed with the aforementioned environmental constants.

Natural diets consisted of excised eggplant leaves (*Solanum melongena* L.,"Classic" variety) of potted plants grown and maintained at the University of Florida,

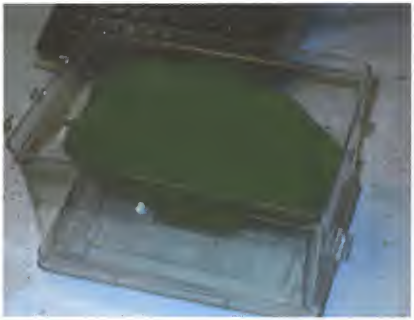

Figure 3-1. Rearing chamber for tobacco hornworm and Colorado potato beetle larvae used in the artificial and excised leaf diet tests. Hardware cloth stage supporting the leaf allowed for easy clean up and minimized disease problems by preventing larvae from coming in contact with fecal material (paper liner not shown).

Department of Entomology and Nematology green and shade houses. Excised leaves were dipped in solutions of deionized H_2O containing different concentrations of methionine; depending on the experiment and exposed to larvae in the same rearing chambers as the artificial diet trials under the same conditions. Survivorship data were pooled from several different trials for data analysis.

In total, 64 potted eggplants were used for the whole-plant portion of the study. Plants were held in FRIUs under the same conditions as the artificial and excised leaf trials, in 38H x 15D (cm) plexiglas cylinders (Figure 3-2). Four THW neonates were placed on each plant for a total of 64 larvae (16 replicates) per treatment (n_{Total}=256 larvae). The treatment of L-methionine was applied to the test plants (using a hand-held sprayer calibrated to deliver approximately 10 mL of solution to each plant) before the addition of larvae.

Feeding and Development

To test L-methionine on the developmental rates of THW, larvae were exposed to excised eggplant leaves dipped in solutions containing the same concentrations of L-methionine used in the artificial diet trials. Additional treatments of proline (1.0%) and *Bt-kurstaki* (Dipel 86% WP® at 3.5 grams/liter; Bonide, Oriskany, NY) were included as positive and negative controls, respectively. Leaves were scanned photometrically using the CI 203 Area Meter with conveyor attachment (CID, Inc.; Camas, WA) to measure leaf consumption before and after exposure to larvae. The difference in leaf areas resulting from the missing leaf tissue was assumed to be the amount eaten by the developing larvae. Larval head capsule widths were measured at the time of death or the

Figure 3-2. Setup for whole plant studies involving tobacco hornworm. Top and portions of the sides were replaced with fine mesh to allow for airflow and to reduce condensation.

end of the trial (using an Olympus Tokyo Model 213598 stereomicroscope with a optical micrometer) to monitor larval development.

Trials to determine the total amount of L-methionine applied to excised leaves also were included to quantify how much of the amino acid was physically present on leaves at the different concentration levels. Leaves were weighed before dipping into the control (0%) and L-methionine solutions (0.1%-10%), allowed to air dry for 30 min and weighed again. The difference was assumed to be the actual amount of L-methionine residue on the leaf. This value then was used to determine the total amount of L-methionine on the leaf surface of the excised leaves and the amount of L-methionine consumed per gram of leaf material, based on calculations of the physical amount of the compound for each % concentration.

Preference Tests

It was unknown if the additional methionine acted to attract or repel larvae. Neonate larvae were used in the choice tests to determine if there was a preference between the control (deionized H_2O) and the Treatments (1.0% L-methionine). Leaves were obtained from potted plants maintained in the outdoor shade house. The tests consisted of 4 leaf disks (30 mm diameter) dipped into the control solution and placed into the chamber alternately with four leaf disks (30 mm diameter) dipped into the treatment solution and replicated with a total of 10 chambers. Each chamber consisted of a large petri dish (25.0 cm diameter x 9.0 cm depth) lined with a Seitz® filter disk. The filter disk was moistened routinely with deionized H_2O to prevent the leaf disks from desiccation (Figure 3-3). Chambers were held in FRIUs at the same environmental constants described previously. The leaf disks also were scanned photometrically and

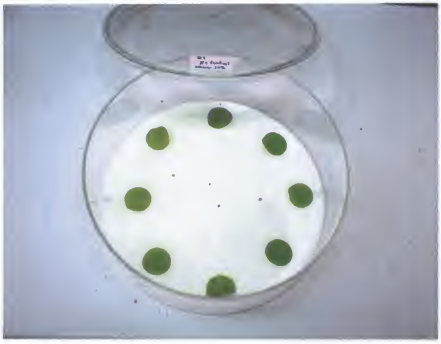

Figure 3-3. Chambers used for tobacco hornworm and Colorado potato beetle preference tests. Two treatments (control and 1.0% L-methionine) were used to determine if any larvae exhibited any preference or avoidance to L-methionine. Treatments were alternated in the chamber and neonates were released in the center of the dish and allowed to search for food. The filter paper in the bottom of the dish was moistened to prevent desiccation of the leaf disks and the test specimens.

larval head capsule measurements made using the same procedures described in the Feeding and Development section.

Data Analysis

Sample sizes of all experiments were chosen according to the guidelines recommended by Robertson and Preisler (1991) for optimal sample size and data analysis. Probit analysis and determination of mean Lethal Concentration (LC_{50}) were performed using PROBIT Version 1.5 (Ecological Monitoring Research Division, USEPA) after Abbott's correction for control mortality (Abbott 1925). Survival data were normalized to the previous value when control mortality was greater than the treatment mortality, to produce a smoother trend line. Statistical analysis was performed on the data using Minitab Version 14 (Minitab, Inc.; State College, PA). Analysis of the data included One-way ANOVA and separation of significant means using Tukey's Multiple Comparison and Pearson Correlation was performed on the choice trial data to examine possible relationships between development and consumption of treated leaf material (Zar 1999). Regression analysis using lest squares were performed on the leaf weights before and after the L-methionine treatment for the equation used to convert % concentration to mg/g plant material (Figure 3-4).

Results

Diets and Survivorship

The artificial diet resulted in 100% mortality of THW larvae for the 3.0% L-methionine to 10.0% L-methionine treatment after only one day of exposure (Figure 3-5). Approximately 80% mortality was observed in the 1.0% L-methionine treatment after 4 days, and 50% mortality for both the 0.3% L-methionine and 0.5% L-methionine

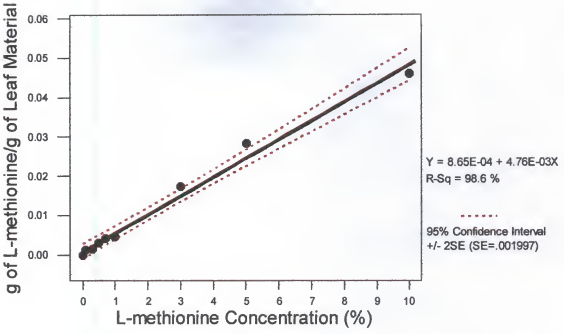

Figure 3-4. Amount of L-methionine present on leaf surface after treatment. Excised leaves were weighed, dipped into various concentrations of L-methionine, allowed to dry, and then re-weighed. Difference assumed to be the amount of L-methionine remaining on leaf surface ($T=22.43$, and $P<0.001$).

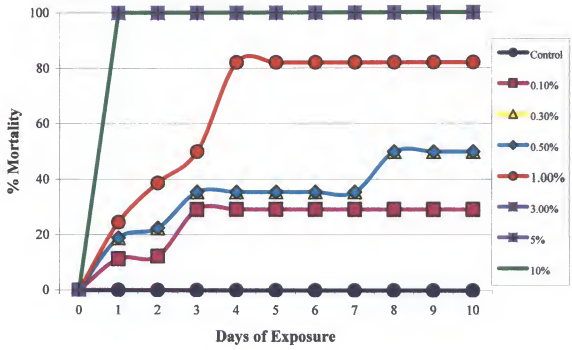

Figure 3-5. Mortality of tobacco hornworm larvae exposed to various concentrations of L-methionine (n_{Total}=480) in artificial diet. Data were adjusted using Abbott's formula to account for control mortality. Note the overlap in trend lines for the 3.0% L-methionine-10.0% L-methionine concentrations after Day 1 and the 0.3% L-methionine and 0.5% L-methionine treatments from Day 1 to Day 10.

treatment after 10 days of exposure. The 0.1% L-methionine concentration had lowest larval mortality with approximately 30% observed for the trial.

The excised leaf trials exhibited higher mortality rates associated with the treatments than did the artificial diet trials. Again, complete mortality was observed with the 3.0% L-methionine thru 10.0% L-methionine concentrations after 1 day of exposure (Figure 3-6). Greater than 90% in the 0.5% L-methionine and 1.0% L-methionine treatments, followed by 80% mortality in the 0.3% L-methionine treatment, and greater than 60% mortality occurred in the 0.1% L-methionine treatment after 8 days.

Whole plant trials produced results similar to the excised leaf trials with greater than 90% larval mortality observed with the 1.0% L-methionine treated plants after 14 days (Figure 3-7). Mortalities exceeding 20% and 60% were observed for the 0.1% L-methionine and 0.5% L-methionine treatments, respectively, during the same time interval.

PROBIT analysis of a sample size of n_{Total}=1,540 for 7 treatments (0.1% L-methionine, 0.3% L-methionine, 0.5% L-methionine, 1.0% L-methionine, 3.0% L-methionine, 5.0% L-methionine and 10.0% L-methionine) revealed an overall LC_{50} of 0.66% (32.3 mg/g leaf material) concentration for the artificial diet and 0.074% (4.39 mg/g leaf material) concentration for the natural diet after 9 days of exposure (Figure 3-8). The LC_{50} for the THW exposed to artificial diet was approximately half the value of that for the natural diet for the 24 to 72 hour exposure period. The LC_{50} for the artificial diet of 1.08% (52.3 mg/g leaf material) for 24 h dropped to 1.0% (48.5 mg/g leaf material) after 48 h and to 0.57% (28.0 mg/g leaf material) after 72 h. As for the natural diet, the LC_{50} of 0.53% (26.1 mg/g leaf material) was found to be lower than the artificial

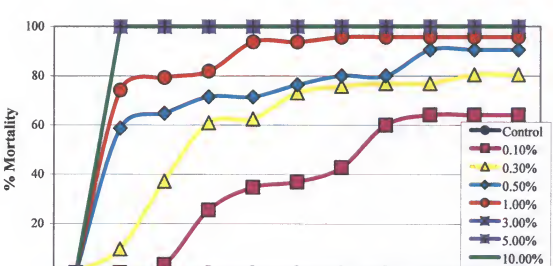

Figure 3-6. Mortality of tobacco hornworm larvae exposed to various concentrations of L-methionine (n_{Total}= 1,540) on excised eggplant leaves. Data were adjusted using Abbott's formula for control mortality. Note the overlap in trend lines for the 3.0% L-methionine-10.0% L-methionine concentrations after Day 1.

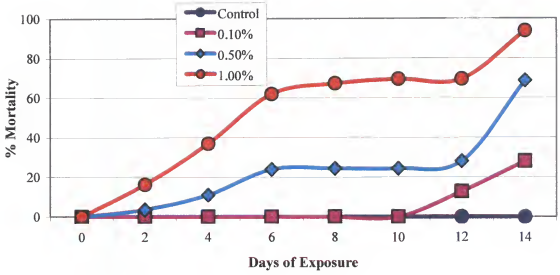

Figure 3-7. Survivorship of tobacco hornworm larvae exposed to various concentrations of L-methionine (n_{Total}=256) on whole plants. L-methionine was applied using a hand-held sprayer in the amount of 10 mL/treatment. Data were adjusted using Abbott's formula for control mortality.

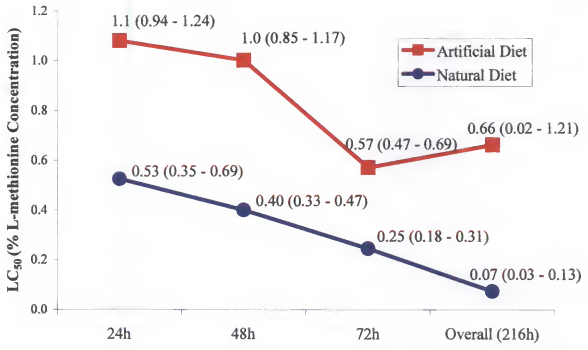

Figure 3-8. Concentrations (%) of L-methionine required for the mortality of 50% of test population of tobacco hornworm after 9 days exposure (n_{Total}=1,540; n=180 for 3.0% L-methionine – 10.0% L-methionine, n=200 for remainder). Number range following value is the 95% confidence limits. Determination of LC_{50} was performed using PROBIT Version 1.5 (Ecological Monitoring Research Division,

diet at 24 h and dropped to 0.4% (19.9 mg/g leaf material) at 48 h and 0.25% (12.8 mg/g leaf material) after 72 h exposure. Overall, the LC_{50} at the end of the experiment for the natural diet was well below the value for the artificial diet, with close to a 90% reduction.

Feeding and Development

Mortality of THW for the developmental tests ranged from approximately 30% for the 0.1% L-methionine treatment and over 40% for the proline treatment (Figure 3-9). Complete mortality for the 0.3% L-methionine occurred after 7 days while the 0.5% L-methionine treatment took only 5 days. The *Btk* treatment mortality was similar to the 0.7% L-methionine and 1.0-%L-methionine treatment, resulting in 100% mortality after 1 day of exposure to the amino acid. Both the mean head capsule width and amount of leaf material consumed showed significant differences between treatments, with the control, 0.1% L-methionine and proline treatments being different that the remaining treatments (Figures 3-10 and 3-11).

Preference Tests

The amount of control and 1.0% L-methionine leaf tissue consumed during the preference tests were found not to be statistically different (Figure 3-12). In addition to the amount of leaf material consumed between treatments not being different, the mean head capsule width (*i.e.*, development) showed a correlation with the amount of control diet consumed (Pearson Correlation Coefficient 0.885, $P<0.001$) while no correlation to the Treatment diet consumed (Pearson Correlation Coefficient 0.630, $P=0.051$) (Figure 3-11).

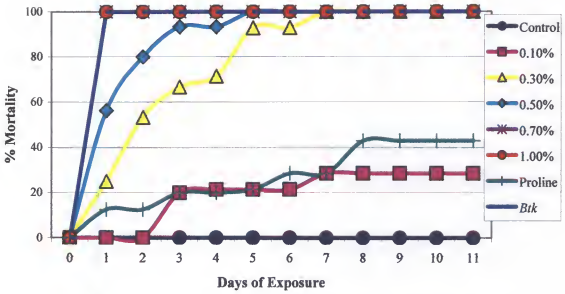

Figure 3-9. Mortality of tobacco hornworm larvae exposed to various concentrations of L-methionine (n_{Total}= 160) on excised eggplant leaves for feeding and development trials. Proline (1.0%) and *Btk* were included for comparison as positive and negative controls. Data were adjusted using Abbott's formula for control mortality. Note the overlap in the 0.7% L-methionine, 1.0% L-methionine and *Btk* treatments at Day 1.

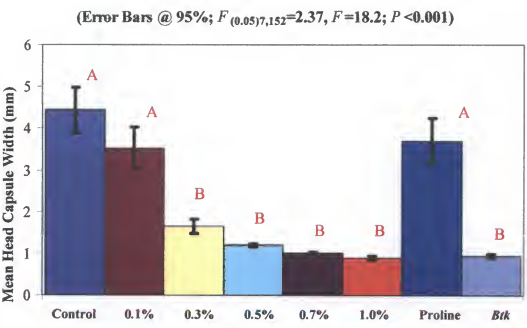

Figure 3-10. Mean head capsule widths of tobacco hornworm larvae exposed to excised eggplant leaves treated with various concentrations of L-methionine (n_{Total}=320). Proline (1.0%) and *Btk* were included for comparison as positive and negative controls. Error bars denote 2 SE. Bars within treatments having the same letter are not statistically different (Tukey's MST, P<0.001).

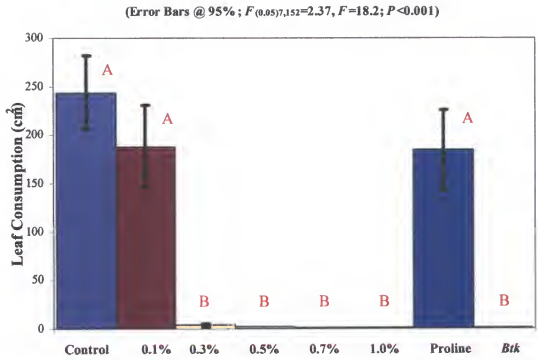

Figure 3-11. Total leaf area consumed by tobacco hornworm larvae exposed to excised eggplant leaves treated with various concentrations of L-methionine (n_{Total}=320). Proline (1.0%) and *Btk* were included for comparison as positive and negative controls. Error bars denote 2 SE. Bars within treatments having the same letter are not statistically different (Tukey's MSTP, P<0.001).

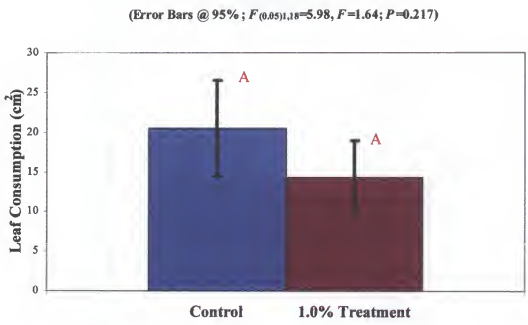

Figure 3-12. Mean leaf consumption by tobacco hornworm in the preference tests. Error bars denote 95% SE, and treatments were found not to be statistically different. However, there was correlation between the control diet consumed and mean head capsule width (Pearson Correlation Coefficient 0.885, P=0.001) while no correlation was found between the Treatment diet consumed and mean head capsule width (Pearson Correlation Coefficient 0.630, P=0.05).

Discussion

The initial studies involving the high concentrations of L-methionine (i.e., 3.0-10.0%, which are outside the range normally encountered in nature) showed that a concentration of 1.0% L-methionine was sufficient enough to provide good control of THW larvae reared on both artificial and natural diets. The 0.1%L-methionine concentration remained similar to that of the control for developmental and feeding trials (Figure 3-9), indicating a level of methionine that can be tolerated to some extent, as seen in the low mortality of this treatment. This is in stark contrast to the mortality seen in the excised leaf trials in which the same concentration had over 60% mortality (Figure 3-6). One possible explanation could be the amount of L-methionine present on the leaf disk being low enough and ingested at a slower rate than that of the whole leaf, which was left in the chamber with the larvae until the leaf was either completely consumed or too wilted for the larvae to ingest.

The preference tests did show some preference towards control leaf disks over the 1.0%L-methionine treated disks as seen in the correlation analysis of the diet consumed and the mean head capsule width of the larvae. Despite the lack of a statistical difference between the amount consumed, the larvae could have fed on the treated disks and then switched to the control disks based on a physiological cue. It is unclear if THW larvae possess specialized sensory structures to detect amino acids like those found in other Lepidoptera (Beck and Henec 1958; Dethier and Kuch 1971; Schoonhoven 1972), but the possible switch from the methionine rich treatment to the control leaf disks does indicate some sort of mechanism for detection. Del Campo and Renwick (2000) found THW larvae were induced to feeding on plants outside of their normal diet when the plants

were treated with an extract from potato foliage suggesting induced host preference, attraction, and dependence on this compound in the extent of sustained feeding and development. A combination of sensory structures may be involved for the detection of specific amino acids and host plant compounds, which may explain the selection of methionine depleted host plants to avoid problems with the CAATCH1 system present in the midgut of the THW.

The difference in the LC_{50} for the artificial and natural diets was striking considering the concentrations were the same. One possible explanation is the L-methionine on the natural diet was more readily available than that found in the artificial diet. With the artificial diet, the L-methionine is presumably spread throughout the diet and would therefore take longer for the THW to ingest enough to adversely affect the CAATCH1 system. In contrast, the L-methionine was found on the surface of the leaf in higher concentrations than that of the artificial diet and was also freely available once ingested. Thus, larvae were exposed to a higher concentration of L-methionine with less work to digest, resulting in lower survivorship in the same period of time.

The 1.0%L-methionine concentration had the same mortality, feeding and developmental rates for THW, as did the *Btk* treatments (Figure 3-9). The 0.3% L-methionine, 0.5% L-methionine and 0.7% L-methionine treatments were virtually the same for mortality (Figure 3-9), developmental rate (Figure 3-10) and total leaf material consumed (Figure 3-11) and statistically the same as the 1.0% L-methionine concentration and the *Btk* treatment. The similar mortality rate observed for the higher concentrations of L-methionine and *Btk* is encouraging considering the resistance to *Bt* seen in many insect species because of reduced receptor activity and binding (Bills *et al.*

2004; Nester *et al.* 2002). Resistance in insects involves a variety of mechanisms and many are the result of a combination of different pesticide classes. The CAATCH1 system is one that could be used in cases where the only alternative is by adding more pesticides or at higher rates to break resistance. Further research is needed to determine compatibility of the different *Bt* insecticides and L-methionine with each other for cases in which *Bt* resistance is observed in natural populations. Given the safety of L-methionine and the shorter time required for 100% mortality (when compared to *Btk* results of this study), this compound could represent a viable alternative for pesticides currently used in the management of the THW.

CHAPTER 4
EFFECTS OF L-METHIONINE ON SURVIVAL AND DEVELOPMENT OF THE COLORADO POTATO BEETLE, *Leptinotarsa decemlineata*, UNDER LABORATORY CONDITIONS

Introduction

Leptinotarsa decemlineta (Say) (Coleoptera: Chrysomelidae), the Colorado potato beetle (CPB), is considered an economic pest throughout North America. The larvae and adults of the CPB feed on a wide variety of solanaceous crop plants and are responsible for $150 million in losses and control related costs (Durham 2000). To further complicate matters, the CPB is resistant to numerous pesticides, including various pyrethroids and carbamates (Bills *et al.* 2004). Historically, CPB management relied heavily on chemical control methods that led to the development of resistance to different pesticides in several areas of the eastern United States (Forgash 1985; Gauthier *et al.* 1981). Control of CPB without the use of chemicals is further complicated given the species ability to develop resistance and the limitations on the use of resistant varieties of potato (Ragsdale and Radcliffe 1999). The use of plant varieties that are resistant to CPB and other pests also run the risk of developing tolerance to chemical pesticides in other pest species (Sorenson *et al.* 1989). Despite the success of *Bacillus thuringiensis-tenebrionis* (*Btt*) and the biocontrol agents *Podisus maculiventris* Say (Hemiptera: Pentatomidae) and *Edovum puttleri* Grissel (Hymenoptera: Eulophidae), more biorational alternatives are necessary for controlling CPB to prevent yet another devastating threat to the potato industry because of this insect's ability develop resistance and overcome control methods (Boucher 1999; Ferro 1985; Tipping *et al.* 1999). This makes the CPB

an excellent candidate for the evaluation of L-methionine as a possible means of controlling this devastating pest.

Because little information is available on the insecticidal properties of L-methionine, several baseline experiments were necessary to determine what concentrations of this amino acid to test. Therefore, it was necessary to test L-methionine and CPB interaction in a variety of ways including survivorship of both larvae and adults, development of larvae when exposed to different concentrations of the amino acid, and preference tests. The purpose of this portion of this study was to conduct bioassays to determine if exposure to L-methionine was detrimental to the survival and development of the CPB and to determine if L-methionine could be used to control this species.

Materials and Methods

Eggs of CPB were obtained under UDSA permit from the insectary of the New Jersey Department of Agriculture and held in 26.4L x 19.2W x 9.5H (cm) clear plastic boxes with a hardware cloth (to facilitate cleaning) and held at 27° C, 60% relative humidity and 16L/8D photoperiod in FRIUs (Figure 3-1). Excised eggplant leafs were placed in the chambers with the neonates and they were allowed to feed for 2 days after eclosion before being transferred to experiments. A camel hair brush was used for transferring the neonates to minimize the risk of damaging the larvae.

Survivorship

Larvae and adults of the CPB were tested in preliminary experiments with the highest concentration (1.0% L-methionine (wt/wt)) observed in tests done on the THW in the previous chapter. The diet for the larvae and adults consisted of excised eggplant leaves (*Solanum melongena* L.,"Classic" variety (Family: Solanaceae)) from plants grown and maintained at the University of Florida, Department of Entomology and

Nematology green and shade houses. Excised leaves were dipped in solutions of deionized H_2O containing different concentrations of methionine and held in the clear plastic boxes and held at the aforementioned environmental conditions (Figure 3-1). Additional treatments of proline (1.0%) and *Bt-tenebrionis* (Novodor® FC @12.4 mL/L; Valent Biosciences, Libertyville, IL) were included as positive and negative controls, respectively. Survivorship data were pooled from several different trials for data analysis.

Feeding and Development

To test L-methionine on the developmental rates of CPB, larvae were exposed to excised eggplant leaves dipped in different concentrations of L-methionine under the same conditions as the survivorship trials. Additional treatments of proline (1.0%) and *Btt* were included as positive and negative controls, respectively. Leaves were scanned photometrically using the CI 203 Area Meter with conveyor attachment (CID, Inc., Camas, WA) before exposure to the larvae and measuring after leaf consumption. The difference in leaf areas resulting from the missing leaf tissue was assumed to be the amount eaten by the developing larvae. Larval head capsule widths were measured at the time of death or the end of the trial (using an Olympus Tokyo Model 213598 stereomicroscope with an ocular micrometer) as an evaluation of larval development.

Preference Tests

It was unknown if the additional methionine acted to attract or repel larvae. Neonate larvae were used in the choice tests to determine if there was a preference between the Control (deionized H_2O) and the treatments (1.0% L-methionine). Leaves were obtained from potted plants maintained in the outdoor shade house. The tests

consisted of 4 leaf disks (30 mm diameter) dipped into the Control solution and placed into the chamber alternately with four leaf disks (30 mm diameter) dipped into the treatment solution and replicated with a total of 10 chambers. Each chamber consisted of a large petri dish (25.0 cm diameter x 9.0 cm depth) lined with a Seitz® filter disk. The filter disk was moistened routinely with deionized H_2O to prevent the leaf disks from desiccation (Figure 3-3). Chambers were held in FRIUs at the same environmental constants described previously. The leaf disks also were scanned photometrically and larval head capsule measurements made using the same procedures described in the Feeding and Development section.

Data Analysis

Sample sizes of all experiments were chosen according to the guidelines recommended by Robertson and Preisler (1991) for optimal sample size and data analysis. Probit analysis and determination of mean Lethal Concentration (LC_{50}) were performed using PROBIT Version 1.5 (Ecological Monitoring Research Division, USEPA) after Abbott's correction for control mortality (Abbott 1925). Survival data were normalized to the previous value when control mortality was greater than the treatment mortality, to produce a smoother trend line. Statistical analysis was performed on the data using Minitab Version 14 (Minitab, Inc.; State College, PA). Analysis of the data included One-way ANOVA and separation of significant means using Tukey's Multiple Comparison and Pearson Correlation was performed on the choice trial data to examine possible relationships between development and consumption of treated leaf material (Zar 1999).

Results

Survivorship

Mortality of CPB larvae on treated excised eggplant leaves ranged from approximately 20% for the 0.1% L-methionine treatment after 4 days, 80% mortality for the 0.3% L-methionine treatment after 8 days of exposure and 100% for the remaining concentrations with the highest dose of 1.0% L-methionine exhibiting complete control of CPB in 3 days post treatment (Figure 4-1). Some mortality (50%) was observed for the proline (1.0%) treatment while the *Btt* larval treatment mortality was similar to the 1.0% L-methionine treatment, resulting in 100% mortality after 5 days.

PROBIT analysis of a sample size of n_{total}=1,320 for 6 treatments (Control), 0.1% L-methionine, 0.3% L-methionine, 0.5% L-methionine, 0.7% L-methionine and 1.0% L-methionine) revealed an overall LC_{50} of 0.218% concentration for the CPB after 8 days of exposure (Figure 4-2). The LC_{50} of 2.9% for 24 hours dropped to 1.1% after 48 hours and to 0.22% after 72 hours.

Feeding and Development

Mean head capsule widths between treatments were found to be statistically different (Figure 4-3). Four distinct groups were observed, with the Control, 0.1% L-methionine and proline treatments forming the first group. The second group of proline and 0.5% L-methionine were statistically the same and likewise the third group of the 0.3% L-methionine, 0.5% L-methionine, and 0.7% L-methionine treatments. The final group of *Btt* and 1.0% L-methionine treatments was statistically different from all other treatments.

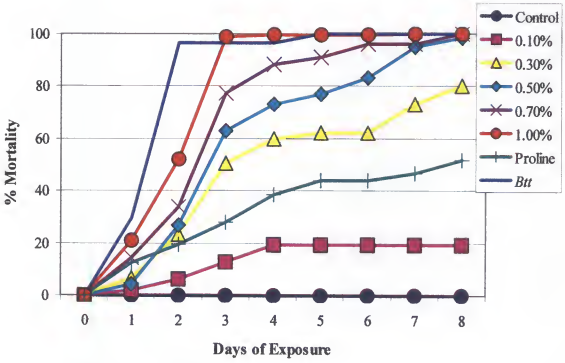

Figure 4-1. Mortality of Colorado potato beetle larvae exposed to excised eggplant leaves treated with various concentrations of L-methionine (n_{Total}=560). Proline (1.0%) and *Btt* were included for comparison as positive and negative controls. Data were adjusted using Abbott's formula for control mortality.

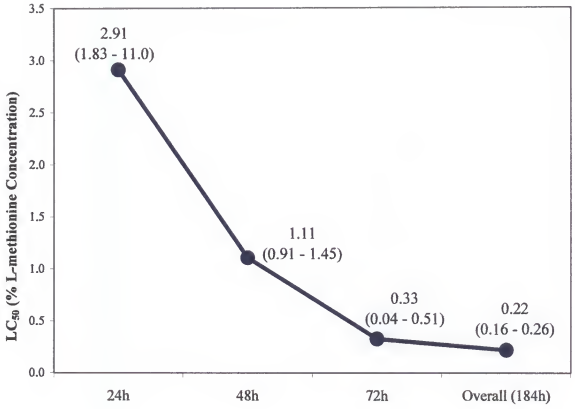

Figure 4-2. Concentrations (%) of L-methionine concentrations required for the mortality of 50% of the test population of Colorado potato beetle after 8 days exposure (n_{Total}=220). Number range following value is the 95% confidence limits. Determination of LC_{50} was performed using PROBIT Version 1.5 (Ecological Monitoring Research Division, USEPA), including Abbott's

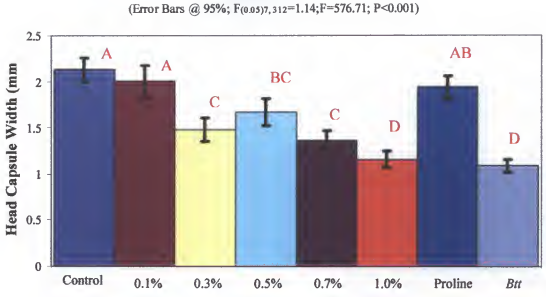

Figure 4-3. Mean head capsule widths of Colorado potato beetle larvae exposed to excised eggplant leaves treated with various concentrations of *L*-methionine (n_{Total}=320). Proline (1.0%) and Bt were included for comparison as positive and negative controls. Error bars denote 2 SE. Bars within treatments having the same letter are not statistically different (Tukey's MST, P<0.001).

Feeding rates of CPB also were found to be statistically different among treatments (Figure 4-4). Three distinct groups were observed with the first group containing the Control and 0.1% L-methionine treatments while the second group of the 0.1% L-methionine and 0.3% L-methionine, treatments were found to be statistically the same. The 0.5% L-methionine, 0.7% L-methionine, 1.0% L-methionine and *Btt* treatments were statistically different from the other groups. Overlap occurred with the proline treatment across all groups indicating no statistical difference with the rest of the treatments.

Preference Tests

The amount of Control and 1.0% L-methionine leaf tissue consumed during the preference tests was found not to be statistically different (Figure 4-5). In addition, the mean head capsule width (*i.e.*, development) showed no relationship with either treatment based upon the low correlation coefficients.

Discussion

The 1.0% L-methionine concentration produced the same larval mortality, feeding and developmental rates for CPB, as did the *Btt* treatments (Figures 4-1, 4-3, and 4-4). The 0.3% L-methionine, 0.5% L-methionine and 0.7% L-methionine treatments took 4 days longer for complete control (Figure 4-1), but were statistically different for the developmental rates for the same treatments (Figure 4-3). As was the case with the THW survivorship, the 0.1% L-methionine concentration was not different from that of the Control. This may indicate a threshold of methionine that can be tolerated by the THW, and CPB to some extent, evidenced by the low mortality observed for this treatment.

The Preference tests did not indicate any preference of leaf disks with or without L-methionine. The high mortality (90%) of the CPB larvae could be explained by a

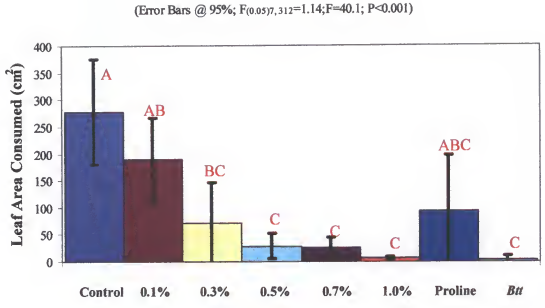

Figure 4-4. Total leaf area consumed by Colorado potato beetle larvae exposed to excised eggplant leaves treated with various concentrations of L-methionine (n_{Total}=320). Proline (1.0%) and *Btt* were included for comparison as positive and negative controls. Error bars denote 2 SE. Bars within treatments having the same letter are not statistically different (Tukey's MST, P<0.001).

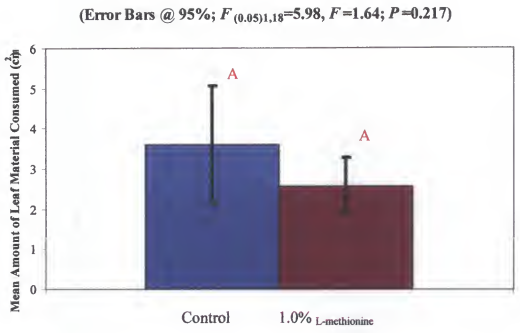

Figure 4-5. Mean leaf consumption by Colorado potato beetle in the preference tests. Error bars denote 95% SE, and treatments were found not to be statistically different. No correlation between either Control or Treatment Diet consumed and mean head capsule width was found (Pearson Correlation Coefficient 0.466, P=0.175 and 0.665, P=0.036, respectively).

combination of the early consumption of the treated disks and mortality occurring after 48 hours, when a lower concentration is required for mortality. The larvae could have fed on the treated disks and then switched to the Control based on a physiological cue. Mitchell (1974) and Mitchell and Schoonhoven (1974) examined the taste receptors of CPB and found physiological and behavioral responses to some amino acids, mainly gamma aminobutyric acid (GABA) and alanine. They discussed the possibility that host selection in solanaceous plants may have been the result of these chemosensory structures and the concentration of amino acids in the leaves. It should be noted that both studies excluded methionine and no electrophysiological data were collected on the response of CPB to this amino acid. This is not surprising considering the fact that the diet of the CPB is low in methionine and therefore would not be a candidate for the inclusion in feeding stimulatory studies (Cibula *et al.* 1967). It is unknown if these sensory structures can detect methionine and possibly act as a means to avoid plant material high in this amino acid. This appears to be contradicted by the data in Figure 4-5, in which there was no difference between the treatments. The larvae feeding on the Control treatment, consuming the majority and then moving to the 1.0% L-methionine treatment, could explain the lack of difference.

There are some differences between some of the Feeding and Development treatments should be noted. The mean head capsule of the larvae in the 0.5% L-methionine treatment was higher than the 0.3% L-methionine treatment while the amount of leaf material consumed for the same treatment were the same indicating another factor involved with the greater head capsule width. The differences could be the result of the larger size of females and possibly could have included more females.

The higher concentrations of L-methionine that produced mortality similar to the *Btt* is encouraging considering the occurrence of resistance to this compound seen in many pest insect species because of reduced receptor activity and binding (Bills *et al.* 2004; Nester *et al.* 2002). Resistance in insects involves a variety of mechanisms and many are the result of exposure to a combination of different pesticide classes. The Methionine-CAATCH1 system could be exploited in cases where the only alternative is applying different pesticides or using higher rates to break resistance. Further research is needed to determine compatibility with *Bt* and L-methionine for cases in which resistance is observed in natural populations. Given the safety of L-methionine and the shorter time required for 100% mortality (when compared to *Btt*), this compound could represent a new biorational tool for the management of the CPB.

CHAPTER 5
EFFECTS OF L-METHIONINE ON SURVIVAL AND DEVELOPMENT OF THE YELLOW FEVER MOSQUITO, *Aedes aegypti*, UNDER LABORATORY CONDITIONS

Introduction

Integrated Pest Management practices are not restricted to agricultural pests. Medically important insect pests are responsible for epidemics that have changed the course of human existence, from bubonic plague spread by the Oriental rat flea (*Xenopsylla cheops* Rothschild (Siphonaptera: Pulicidae)), to malaria carried by anopheline mosquitoes. One medically important species that has had a significant impact on human existence is the yellow fever mosquito (YFM), *Aedes aegypti* (L.) (Diptera: Culicide). This cosmopolitan species is found worldwide and is the primary vector for human dengue and yellow fever despite concerted efforts at eradication in the United States (Womack, 1993). In the United States alone, upwards of 150,000 lives were lost to yellow fever in the period starting in the late 18th century and into the early 20th century (Patterson, 1992). However, because of the development of a vaccine, yellow fever has been replaced by Dengue which is now second only to malaria as a worldwide threat (Gubler, 1998). Because Dengue fever is also vectored by the YFM, it poses a risk by affecting tens of millions of people worldwide (Gubler and Clark, 1995).

The inclusion of the YFM in this study was an effort borne of curiosity because of the lack of knowledge of the CAATCH1 system in other insects and the availability of specimens for study. Mosquito larvae are particulate feeders and have dietary

requirements of methionine in the amounts of 0.0007mg/ml for the YFM. This amino acid also is considered essential for other species of mosquito in untraceable (in those studies) amounts (Chen, 1958; Singh and Brown, 1957). Given the high alkalinity found in the midgut of the YFM as well as other mosquito species, this physiological condition indicates the possibility of the presence of the CAATCH1 system in larval mosquitoes (Dadd, 1975).

The purpose of this portion of the study was to examine the survival and development of YFM larvae exposed to water treated with excess L-methionine (adults were not tested given the feeding nature). In addition to L-methionine, other amino acids were tested in an effort to see if their response (*i.e.,* survivorship) was similar CAATCH1 responses to methionine found by Feldman *et al.* (2000).

Materials and Methods

Bioassay

The bioassay experiments consisted of six treatments (control, 0.1%, 0.3%, 0.5%, 0.7% and 1.0%) each with four replicates. Both L-methionine and D-methionine were tested along with proline, Beta-alanine and L-leucine to examine the other amino acids that were found to be reactive to the CAATCH-1 system (Feldman *et al.*, 2000). *Bt-israeliensis* (Aquabac® @ a rate of 2.3 mL/m^2; Biocontrol Network, Brentwood, TN) and proline also were included in some trials of L-methionine to allow for comparison of both positive and negative effects. Amino acids were weighed using a Denver Instruments Co. XD2-2KD digital scale and added to glass quart jars containing 500ml of deionized H_2O. Concentrations were based on the proportion of 1g/100ml for a 1% solution and for corresponding concentrations. Solutions were allowed to sit at room

temperature (23°C) to permit the amino acid to fully dissolve before the addition of the larvae. An additional trial of L-methionine buffered with Tris to a pH of 7.0 using a Fisher Scientific Accumet pH 900 was conducted to determine if mortality was attributed to a change in pH or exposure to the L-methionine.

Larvae of YFM (third instar) were obtained from the mosquito colony maintained at the Department of Entomology and Nematology, University of Florida. Larvae were transferred to the treatment jars using a camel hair, with 10 larvae per replicate for a total of 40 larvae/treatment and n_{Total}=240 for each amino acid bioassay experiment (Figure 5-1). Approximately 0.5g of finely ground fish food was added to serve as a larval food source and nylon window screen was used to cover the tops of the jar to prevent the escape of any emerged adults. Jars were held at 23°C on a dedicated laboratory bench top for approximately one week. The numbers of larvae surviving were recorded each day.

Growth and Development

This experiment used the same Materials and Methods as the bioassay portion with the exception of neonate larvae instead of 3rd instars. Eggs were placed in a tray of water and held at 23°C for 2 days after eclosion. Neonates were removed using a camel hair paintbrush and placed into each jar, with 10 larvae per replicate for a total of 40 larvae/treatment (n_{Total}=240). Larval exuviae or dead larvae were removed and used to examine growth rates by measuring the head capsules. Larvae head capsule widths were measured (using an Olympus Tokyo Model 213598 stereomicroscope with an ocular micrometer) as an evaluation of larval development.

Figure 5-1. Bioassay setup for yellow fever mosquito larvae exposed to various concentrations of amino acids and *Bti*. Jars contained 500mL of solution and were covered with screen to prevent the escape of emerging adults.

Data Analysis

Sample sizes of all experiments were selected according to the guidelines of Robertson and Preisler (1991) for optimal sample size and data analysis. Probit analysis and determination of mean Lethal Concentration (LC_{50}) were performed using PROBIT Version 1.5 (Ecological Monitoring Research Division, USEPA) after Abbott's correction for control mortality (Abbott 1925). Probit analysis was performed on different concentrations (0.1%, 0.3%, 0.5%, 0.7% and 1.0%) of L-methionine, Tris-buffered L-methionine, D-methionine, Beta-alanine, proline and L-leucine for 24, 48, 72 and 168 hours (the end of the trials). Survival data were normalized to the previous value when control mortality was greater than the treatment mortality, to produce a smoother trend line. Statistical analyses were performed on the data using Minitab Version 12. Analysis (Minitab, Inc; State College, PA) of the data included One-way ANOVA and separation of means using Tukey's Multiple Comparison test (Zar 1999).

Results

Bioassay

Mortality of YFM larvae in both the unbuffered L-and D-methionine trials was similar with low or no mortality, at the 0.1% concentrations (Figures 5-2 and 5-3). The 0.3% concentration had lower mortality with D-methionine (45%) than L-methionine (75%) and greater than 80% mortality for the 0.5% concentration for both isomers. Higher concentrations of both D-and L-methionine forms produced 100% mortality of the larvae within 2 days after treatment.

Greater than 40% mortality was observed for the buffered 0.1% L-methionine concentration with complete mortality for the remaining treatments within 5 days of

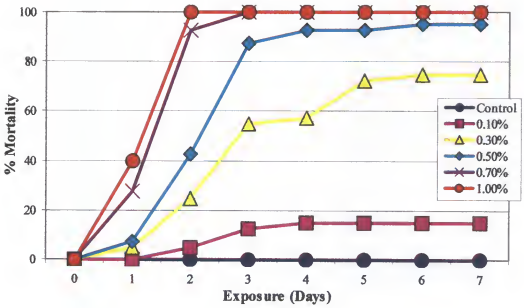

Figure 5-2. Mortality of yellow fever mosquito larvae exposed to various concentrations of L-methionine (n_{Total}=240). Data were adjusted using Abbott's formula for control mortality.

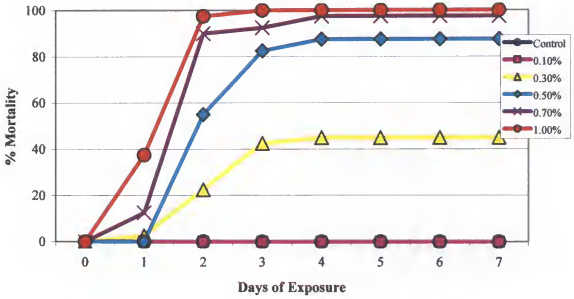

Figure 5-3. Mortality of yellow fever mosquito larvae exposed to various concentrations of D-methionine (n_{Total}=240). Data were adjusted using Abbott's formula for control mortality.

exposure (Figures 5-4). The $1.0\%_{\text{L-methionine}}$ treatment caused 100% mortality after 2 days while the *Bti* treatment took 3 days to reach the same level of control. The proline treatment caused less than 10% mortality.

In contrast to methionine, survival of YFM larvae exposed to proline and L-leucine was higher, with only approximately 20% mortality for the higher 0.7% proline and 1.0% proline concentrations (Figure 5-5) and less than 3% mortality with the highest L-leucine concentration (Figure 5-6). Beta-alanine mortality was similar to the L-methionine treatments with between 75% and 83% mortality for the 0.5% Beta-alanine thru 1.0% Beta-alanine concentrations, respectively, greater than 40% mortality with the 0.3% Beta-alanine, and less than 5% mortality for the 0.1% Beta-alanine concentrations (Figure 5-7).

Growth and Development

Developmental rates of YFM larvae resulted in three distinct groups, with the control and proline treatments, producing virtually identical results; both were statistically different from the 0.1%L-methionine treatment and the remaining L-methionine treatments (Figure 5-8). The *Bti* treatment was statistically the same as the 0.3% L-methionine to 1.0% L-methionine treatments, with very little growth taking place.

Probit analysis for unbuffered L-methionine (n_{Total}=40 for 5 treatments; 0.1%, 0.3%, 0.5%, 0.7% and 1.0%) revealed an overall LC_{50} of 0.19% concentration for the YFM after 7 days of exposure (Figure 5-9). The LC_{50} of 1.2% for 24 hours dropped to 0.41% after 48 hours and to 0.24% after 72 hours. When the L-methionine treatments (same concentrations) were buffered to a pH 0f 7.0, the values dropped to 0.64% for 24

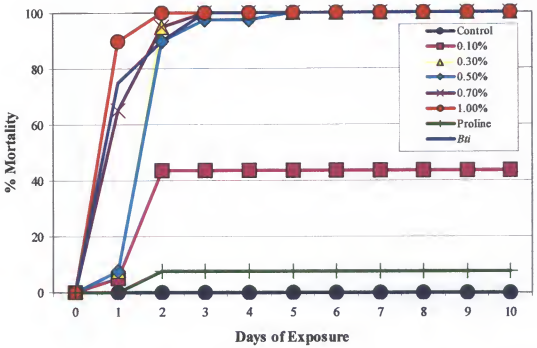

Figure 5-4. Mortality of yellow fever mosquito larvae exposed to various concentrations of Tris-buffered L-methionine (n_{Total}=240). Data were adjusted using Abbott's formula for control mortality. Note the longer exposure because of the bioassay involving neonates instead of 3rd instars. Note the overlap in some of the trend lines on Day 1 with the 0.3% L-methionine and 0.5% L-methionine treatments.

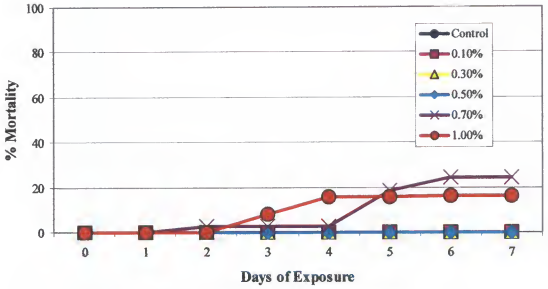

Figure 5-5. Mortality of yellow fever mosquito larvae exposed to various concentrations of Proline (n_{Total}=240). Data were adjusted using Abbott's formula for control mortality. Note the overlap of trend lines for all treatments except the 0.7% L-methionine and 1.0% L-methionine treatments.

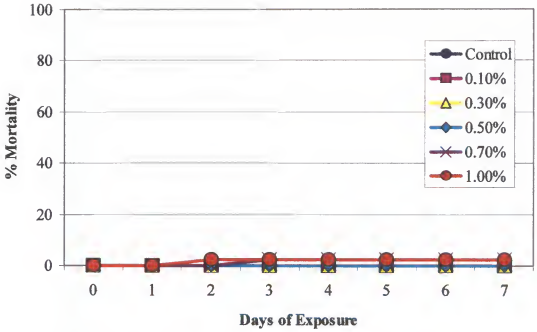

Figure 5-6. Mortality of yellow fever mosquito larvae exposed to various concentrations of L-leucine (n_{Total}=240). Data were adjusted using Abbott's formula for control mortality. Note the overlap in trend lines for all treatments.

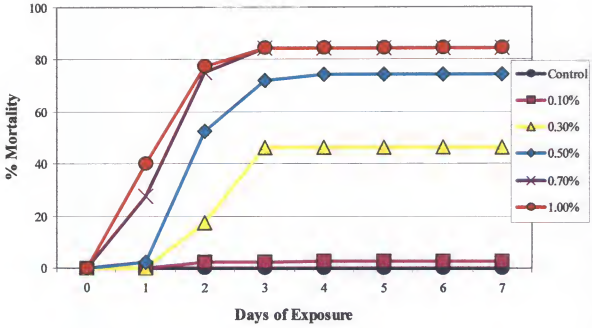

Figure 5-7. Mortality of yellow fever mosquito larvae exposed to various concentrations of Beta-alanine (n_{Total}=240). Data were adjusted using Abbott's formula for control mortality.

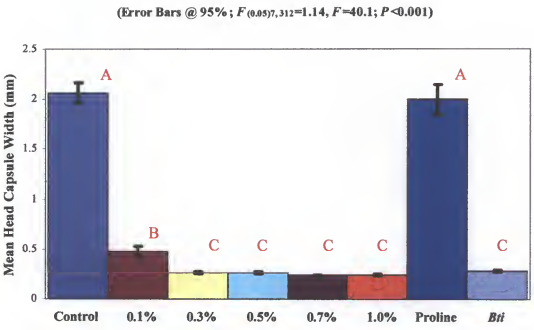

Figure 5-8. Mean head capsule widths of yellow fever mosquito larvae exposed to various Tris buffered (7.0 pH) concentrations of L-methionine (n_{Total}=320). Proline (1.0%) and *Bti* were included for comparison as positive and negative controls. Error bars denote 2 SE. Bars within treatments having the same letter are not statistically different (Tukey's MST, P<0.001).

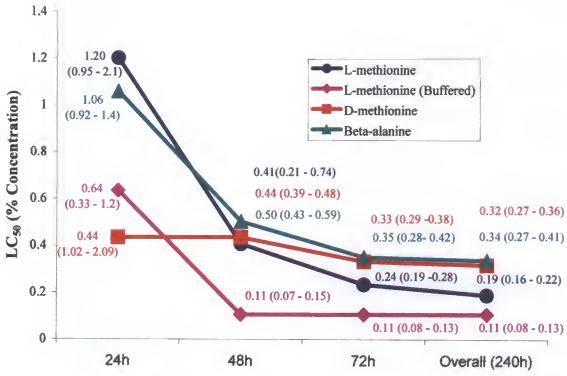

Figure 5-9. Concentrations (%) resulting in 50% mortality (LC_{50}) of yellow fever mosquito larvae exposed to various amino acids after 10 days (n_{Total}=240 for each amino acid). Number range following value is the 95% confidence limits. Proline and L-leucine were also tested but did not exhibit sufficient mortality to allow for Probit Analysis.

hours, and to 0.11% for 48-168 hours and remained constant since the trial lasted longer because of the use of neonates instead of 3rd instars. The D-methionine treatments were similar with 0.44% for 24 and 48 hours, 0.33% for 72 hours and 0.32% after 168 hours. While not as striking as the others, Beta-alanine had a LC_{50} concentration of 1.1% after 24 hours, dropped to 0.5% after 48 hours and leveled off around at 0.35% after 72 and 168 hours. Probit analysis of the Proline and L-leucine treatments was not performed, as the mortality associated with those treatments was too low (Figures 5-5 and 5-6).

Discussion

Although not commonly encountered, the D- form of methionine had virtually the same effect as the L- form on larval mosquito mortality. The D-and L-methionine trials showed that the D- form had lower mortality associated with it than the more reactive L-counterpart. Insects do not commonly use the D- form of amino acids, although D-methionine is metabolized by some orders to a limited extent (Ito and Inokuchi, 1981). The YFM could be an example of this phenomenon.

Because of the nature of the CAATCH1 system in the alkaline midgut, buffering may have acted to increase the effectiveness of the system. Buffering the solutions did result in an increase in mortality, with even the lowest concentration of 0.1% L-methionine exhibiting a two-fold increase with the buffered form (Figure 5-4). Complete mortality was reached sooner with the buffered forms even for concentrations that did not reach 100% in the unbuffered form. In a field setting, the addition of L-methionine would be buffered naturally by the chemical properties of the bodies of water to which it was applied and similar results would be expected.

Jaffe and Chrin (1979) found the adults of YFM females infected with *Brugia*, a filiaral parasite, were depleted of free form methionine because of the infection and were able to make up the difference by converting homocysteine to methionine with a special synthetase. The ability of YFM adults to synthesize methionine from homocysteine may be present in the larvae as well. This could be the result of the lack of methionine in the diet and possible evidence of the CAATCH1 system being present in at least the adult stage. The susceptibility of the larvae to L-methionine also could be the result of overexposure to a compound that is normally not encountered in high concentrations (>0.1%). However, the alkalinity of the particulate feeding larvae and the high mortality to L-methionine suggests that the CAATCH1 system is present and could be exploited in other species with similar midgut characteristics (Dadd, 1975).

The survival of YFM larvae exposed to both Beta-alanine and L-leucine was unusual in that they each had the opposite effect on the YFM larvae. L-leucine was expected to have similar blocking properties as L-methionine based on CAATCH1 research (Feldman *et al*., 2000). Instead, almost no mortality was observed indicating the possibility of another system involved with the transport of this amino acid. Conversely, beta-alanine was not found to be reactive with the CAATCH1 system based on the work of Feldman *et al*. (2000). The unusually high larval mortality associated with this amino acid may be the result of a yet to be discovered midgut property.

The similar mortalities observed for the higher concentrations of L-methionine and *Bti* is encouraging considering the resistance to this compound that has been documented in many insect species because of reduced receptor activity and binding (Bills *et al*., 2004; Nester *et al*., 2002). Resistance in insects involves a variety of

mechanisms and many are the result of a combination of different pesticide classes. The CAATCH1 system is one that could be exploited in cases where the only alternative is applying different or higher rates of pesticides to break resistance. Further research is needed to determine compatibility of *Bti* and L-methionine for cases in which resistance is observed in natural populations. Given the safety of L-methionine and the similar time required for 100% mortality (when compared to *Bti*), this compound could represent a viable alternative to traditional biorational compounds used in the management of the YFM or other susceptible pest mosquito species.

CHAPTER 6
FIELD EVALUATION OF L-METHIONINE AS AN INSECTICIDE

Introduction

The role of methionine in animal systems is well known and only recently understood in plants. Methionine is required for protein synthesis; it is a precursor to several important biochemical compounds including ethylene and polyamines, sulfate uptake and assimilation, and also acts as an activator of threonine-synthase (Giovanelli *et al.* 1980; Droux *et al.* 2000; Bourgis *et al.* 2000; Zeh *et al.* 2001). Recently, research has focused on the transgenic modification of crop plants to overproduce methionine in order to increase their nutritional quality without affecting other biochemical processes (Zeh *et al.* 2001). However, little work has been conducted on the effects of exogenous methionine and it became important to understand the role of externally applied methionine on plant health.

Furthermore, the application of L-methionine to plants exposed to natural conditions presents additional problems in terms of how long the residue remains on the plant. Observations of other experiments using L-methionine revealed the tendency of this compound to crystallize after the aqueous portion evaporated forming a brittle, crusty coating that is easily removed. This coating does not appear to interfere with respiration and transpiration at the concentrations studied (1% and lower). To prevent the loss of L-methionine from the plants in a natural setting, the adjuvant Silwett L-77® (Helena Chemical; Collierville, TN) was included in this portion of the study in an effort to increase residual activity on the plant. Silwet L-77® is a nonionic organosilicate

surfactant that has wetting and spreading properties (Helena Chemicals 2002) and was found to be compatible with solutions of L-methionine.

The objectives for this portion of the study were to examine the effects of a methionine and Silwet L-77® mixture on a crop plant (eggplant) in terms of yield (both fruit weight and total yield) and to evaluate this mixture as an insecticide under natural conditions.

Materials and Methods

Preliminary Investigation of Silwet L-77® and L-methionine

Adult CPBs were obtained from the University of Florida Horticultural Unit, Gainesville and held in 26.4L x 19.2W x 9.5H (cm) clear plastic boxes with a hardware cloth (to facilitate cleaning) and held at 27°C, 60% relative humidity and 16L/8D photoperiod in FRIUs. Twenty-four adults were exposed used in each of the 5 treatments, with 4 replicates per treatment (n_{Total}=120). Adults were used because of the lack of sufficient numbers of larvae to test. Excised leaves were dipped in solutions of deionized H_2O containing different concentrations of methionine and Silwett L-77® (0.5% concentration), 0.1% L-methionine, 0.5% L-methionine, 1.0% L-methionine and controls of deionized H_2O and deionized H_2O +Silwet L-77®. The additional control was to determine the possible insecticidal properties of Silwet L-77® alone and to make sure the addition of this adjuvant did not affect mortality or deter feeding.

Plot Design

Eggplants (*Solanum melongena* L.,"Classic" variety) were grown and maintained at the University of Florida Horticultural Unit, Gainesville, from 18 June to 04 November 2001. Eight, one hundred ft. rows of plants were used for this study, with two rows on each side consisting of buffer rows and four rows in the middle used for the experiments.

Each row contained the 4 treatment plots of 10 plants (control (0% L-methionine), 0.1% L-methionine, 0.5% L-methionine and 1.0% L-methionine in deionized water solutions) in a Latin square design. Plants within treatment plots were spaced 3 feet apart while treatment plots were 9 feet apart. Figure 6-1 shows the diagrammatic representation of the field plot.

Plant Yield

Before beginning the experiment, all developing eggplants were removed from the plants in an effort to standardize the treatments and ensure all eggplant development occurred after the exposure of methionine. Treatments were administered using a KQ 3L CO_2 (Weed Systems, Inc.; Hawthorne, FL) backpack sprayer charged to 30 lbs PSI and a 3-nozzle boom to ensure complete coverage of the plant (Figure 6-2). Each treatment consisted of a 3L application over the 4 representative groups. The adjuvant Silwett L-77® (0.5% concentration) was included to improve the residual effect of the methionine under the field conditions. Plants were sprayed a total of nine times at approximately two-week intervals. Fruits were harvested at various times during the study and were weighed in the field using a Tokyo Electronics hand-held digital scale.

Pest Introduction

Neonate CPB larvae were reared on excised eggplant leaves for two days at 27°C, 60% relative humidity and 16L/8D photoperiod in FRIUs to ensure healthy individuals for the test. Larvae were transferred to the field plants using a camel hairbrush and the branch marked with flagging tape. Introduction was made after the last spray treatment in November. Ten larvae were placed on each plant for a total sample size of 1,600 individuals. Plants were inspected for the next 5 days and larvae encountered noted.

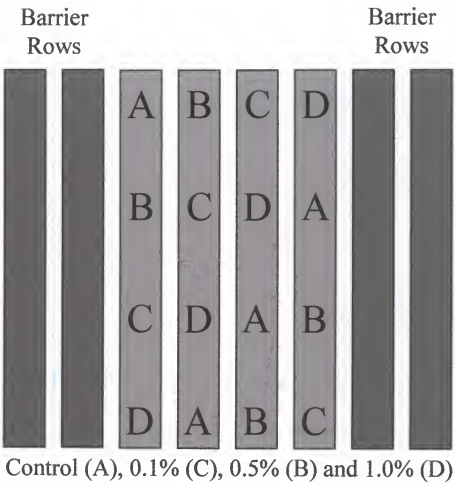

Figure 6-1. Overview of the design layout used to study the effects of L-methionine and Silwett L-77® solutions on yield of eggplant. Rows were four feet apart with individual plants three feet apart and treatments nine feet apart. Each letter represents a group of ten eggplants.

Figure 6-2. Weed Systems, Inc. KQ 3L CO_2 backpack back sprayer used for application of L-methionine and Silwett L-77® solutions. Boom consisted of three nozzles (middle top and end of each arm). In total, 3L were applied per treatment every two weeks from 09 July to 31 August 2001.

Data Analysis

Data from the fruit and the CPB experiments were analyzed with ANOVA using Minitab Version 12. Survivorship of CPB was corrected using Abbott's formula (Abbott 1925) to account for control mortality, mean separation was performed using Tukey's multiple comparison procedure (Zar 1999). Data for both the eggplant weight mean per treatment and also mean number of eggplants per treatment were analyzed using paired t-test.

Results

Effects of L-methionine and Silwett L-77® on CPB Adults Under Laboratory Conditions

Little mortality was observed with the adult CPB at the 1.0% L-methionine concentration (Figure 6-3). The 0.5% L-methionine concentration had the highest mortality of all the treatments at approximately 20% with the other treatments showing no adverse effects after correction for control mortality.

Effects of L-methionine and Silwett L-77® on yield

In total, 735 eggplants were collected during the course of this study from 09 June to 31 August 2001. Mean weight and yield of eggplants between the treatments were not statistically different from each other (Figures 6-4). Control plants produced 195 fruits with a mean weight of 276.9 grams, followed by the 0.1% treatment with 191 fruits at 281.2 grams. The 0.5% and 1.0% treatments yielded 175 and 174 fruits with mean weights of 295.7 grams and 283.6 grams, respectively.

Survival of CPB larvae

No statistical difference in survivorship of CPB larvae was observed between the three treatments for the first day after exposure (Figure 6-5) but treatment differences

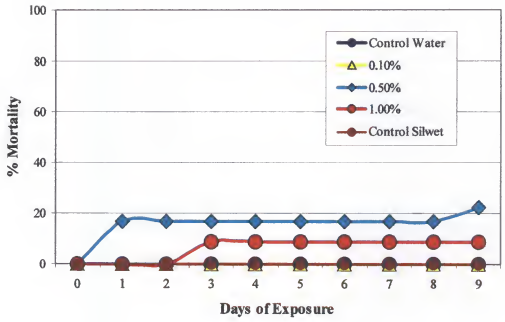

Figure 6-3. Mortality of Colorado potato beetle adults exposed to excised eggplant leaves treated with L-methionine and the adjuvant Silwett L-77® (n_{Total}=120). Data corrected for control mortality using Abbott's formula. Note the overlap in trend lines for the Control treatments and $0.1\%_{L\text{-methionine}}$ treatment.

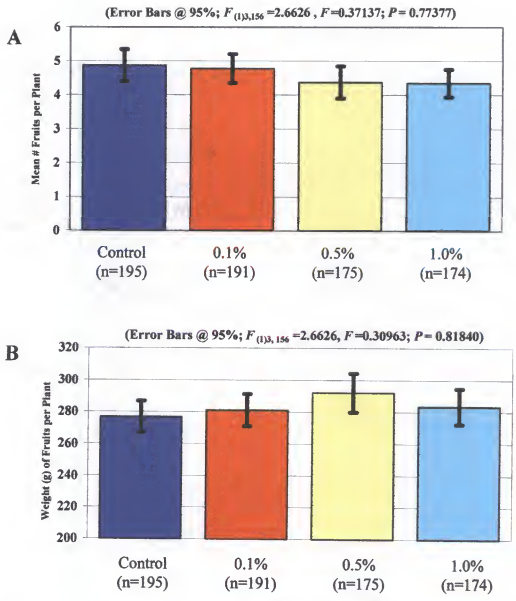

Figure 6-4. Effects of L-methionine and Silwett L-77® on eggplant yield (A) and mean weight in grams of fruit (B) from 09 June to 31 August 2001. Error bars denote 2 SE. There was no statistical difference for either eggplant yield or mean eggplant weight (Tukey's MST, P=0.05).

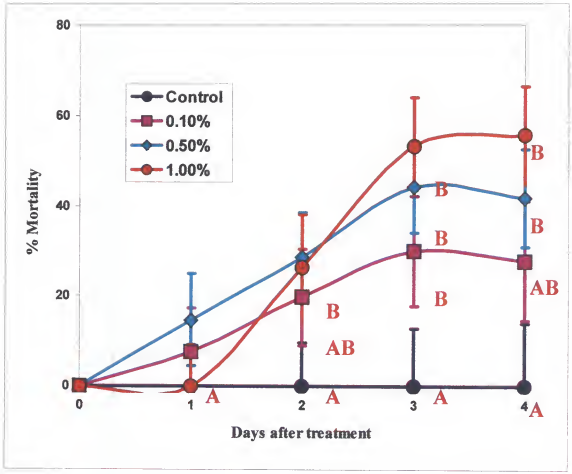

Figure 6-5. Mortality of Colorado potato beetle larvae on eggplants treated with L-methionine and Silwett L-77®. Mortality of larvae corrected using Abbott's formula (Abbott, 1925). Analysis performed on arcsin transformed data. Error bars denote 2 SE. Data points having by the same letter are not statistically different (Tukey's MST, P=0.05)

were observed thereafter. By Day 4 the 1.0% and 0.5% treatment were the only treatments that were statistically different from the control. There was substantial unexplained attrition of CPB larvae in the field for all treatments, which leveled off by Day 3. Data from day 5 was discounted because of the onset of a severe cold front that made it difficult to separate the effects of the weather from the treatments affects.

Discussion

The results of the field studies show that, using conventional application techniques, a mixture of methionine and Silwett L-77® did not appear to affect eggplant yield. Furthermore, the same combination produced substantial control of CPB larvae under natural field conditions after four days. Dahlman (1980) found that L-canavanine, a non-protein amino acid, could be used in the same manner for control of THW on tobacco, but the widespread use of this compound was limited by the cost ($107.85 for 1g L-canavanine versus $3.35 for 1g of L-methionine (Fisher Scientific International 2004)), adverse effect on plant development (Nakajima *et al.* 2001), and toxicity to vertebrates (Rosenthal 1977). Although complete coverage of the plant was not feasible, approximately 2.5 grams to 7.5 grams of L-methionine was applied to the plants in each of the treatment plots. Each plant, based on the amount applied, received approximately $7.5x10^6$ μg for the 1.0% L-methionine treatment, $3.8x10^5$ μg for the 0.5% L-methionine treatment and $2.5x10^4$ μg for the 0.1% $_{\text{L-methionine}}$ treatment. This compares to only 4μg of L-canavanine, which resulted in decreased size, fecundity, and mortality of THW under field conditions (Dahlman 1980). It should be noted that the toxicity of L-canavanine is well documented and has a different mode of action than L-methionine and cannot be

compared directly. However, the cost for the amount of L-canavanine required for large-scale application far exceeds that of the largest amount of L-methionine needed.

Despite the lack of a statistical difference between treatments for both mean weight and mean yield of eggplant, there were some interesting disparities within the data. First, there was an observable difference in mean weight of the eggplants between the treatments and the control. All eggplant weights were greater for the treatments than the control, with the 0.5% L-methionine concentration treatment producing the highest mean eggplant weight. It would appear that excess methionine decreases the number of fruit produced, but those fewer eggplants weighed more. Further research is needed to better understand the differences observed during this study.

The addition of Silwet L-77® did not appear to adversely affect survival of CPB as seen in the preliminary tests on the adults and on the larvae during the field release (Figures 6-3 and 6-5). The low adult mortality observed could be attributed to the ability of this species to stop feeding and fly to a more suitable food source. Because the adults were unable to move to an untreated leaf, they were observed sitting motionless on the underside of the leaves. This was not observed in either of the controls as they were seen actively feeding the majority of the time.

One aspect of this research that was not examined is that of fertility and fecundity of adults exposed to excess amounts of L-methionine. Despite the fact that methionine is used for egg production in many insect species, excess concentrations may act as a deterrent to feeding causing the adults to stop feeding and to seek other food sources. The lag time from the cessation in feeding to finding another food source may be long

enough to significantly lower the fecundity of the females and possibly interfere with other behaviors such as mating.

During the course of this portion of the study, some anecdotal data were collected based on personal observations. Predators (mainly arachnids) were observed on the plants until the end of the experiment. Other insects also were observed feeding on plants after treatments including piercing-sucking insects (i.e., aphids, coreids and cicadellids) with foliage feeders such as caterpillars rarely encountered except found only on control plants. Attempts to control predators *via* manual removal were unsuccessful, and predation may have contributed to the observed decrease in CPB. Because predators were present on all treatments, loss from predation was corrected with the use of Abbott's formula. The presence of natural enemies indicates the selectivity of the L-methionine in the field. The amount of methionine ingested by the predators was probably very small because they fed on other insects not plant material.

Another set of observations on the safety of L-methionine was the exposure of potted eggplants to high (1.0% methionine in distilled H_2O solution). In total, five plants were sprayed daily with the methionine solution and compared to five plants sprayed with water alone for 14 days. The only difference in the plants was the browning of the leaf tips and edges of the methionine sprayed plants. This also was seen in the excised leaf experiments with THW and CPB. A possible reason for this occurrence was the excess sulfur in the methionine might have burned the leaves. As mentioned earlier, the concentration was very high and also applied daily. Applications of the same concentration did not affect the plants in the field plots, indicating that treatments conducted at 2-week intervals would be safe for the plant.

Overall, it appears that L-methionine can be used in a natural setting to control CPB larvae without affecting crop production. The adjuvant Silwett L-77® worked well with L-methionine in controlling CPB larvae but not the adults. The lack of effectiveness on the adults may be attributed to their ability to stop feeding and living off of reserves acquired during the larval stage until suitable food sources can be found. It is unknown if L-methionine, alone or in combination with Silwett L-77® adversely affects fecundity of the adults.

CHAPTER 7
EFFECTS OF L-METHIONINE ON SURVIVAL AND DEVELOPMENT OF SELECTED NONTARGET SPECIES

Introduction

A biorational pesticide is defined as one that is effective against pest species but innocuous to non-target organisms and not disruptive to biological control agents and beneficial species (Stansly *et al.* 1996). To test L-methionine as a potential pesticide and determine if it could be considered biorational, it was necessary to examine the effects of this compound on selected nontarget species that could possibly come into contact with it, either directly while on the plant or indirectly *via* incidental contact or as a host that has come into direct contact with this compound. The species chosen reflect a variety of non-target organisms, mainly those that were shown to be important in controlling some pest species. The pink spotted ladybird beetle, *Coleomegilla maculata* (DeGeer), the mottled water hyacinth weevil, *Neochetina eichhorniae* Warner, and the greenbug parasitoid, *Lysiphlebus testaceipes* (Cresson) all are beneficial insects that have been effective against pests in the state of Florida and also are common and readily available. Each species also represents a different feeding guild (predator, herbivore and parasitoid, respectively) to ensure a thorough examination of the possible effects of methionine as it might be encountered in under natural conditions.

The pink spotted ladybird beetle (PSLB) is an abundant polyphagus species that is known to feed on many lepidopteran and coleopteran pests, including the Colorado potato beetle, in which it was responsible for over 50% of the predation on eggs and early

instars (Andow and Risch 1985; Giroux *et al.* 1995; Griffin and Yeargan 2002; Groden *et al.* 1990; Hazzard *et al.* 1991; Hilbeck and Kennedy 1996; Munyaneza and Obrycki 1998). This species is widespread throughout North America, and has been shown to provide effective biological control in several crop species, including corn, crucifers, tomato and potato (Hoffman and Frodsham 1993). However, the PSLB was found to be susceptible to carbaryl and menthamidophos, the same pesticides used for the control of many aphid species (Hoffman and Frodsham 1993).

Since its introduction into the United States in 1884, water hyacinth (*Eichhornia crassipes* (Mart.) Solms-Laubach) has infested waterways of the southeast that has cost upwards of $2 million to control in Florida alone (Schardt 1987). The mottled water hyacinth weevil (MWHW), native to Argentina, was first released in Florida in 1972 and subsequently to other states and countries in an effort to control water hyacinth (Center 1994). The genus is restricted to feeding on members of Pontederiaceae, with the MWHW feeding mainly on the introduced water hyacinth; it can be found virtually everywhere the host plant is present (Haag and Habeck 1991; Center *et al.* 1998).

The greenbug parasitoid (GBP) is an important natural enemy of many cereal aphids. This species is known for the production of "mummies", the bodies of parasitized aphids that act as a protective case for the developing wasp pupa, and is considered by many to be tolerant to cold temperatures (Elliott *et al.* 1999; Knutson *et al.* 1993; Wright 1995). However, this greenbug parasitoid is an insect and is just as susceptible to pesticides despite the protective case of the immature form (Knutson *et al.* 1993).

The purpose of this portion of the study was to examine the effects of L-methionine on selected nontarget species that are both important in terms of being beneficial in controlling other pest species and also represent different feeding guilds that would come into contact with this compound in different ways (*e.g.*, on prey items, on plant surfaces, hosts of parasitoids).

Materials and Methods

Coleomegilla maculata

Adults were obtained from ENTOMOS, LLC (Gainesville, Florida), and were held in 26.4L x 19.2W x 9.5H (cm) clear plastic boxes with a hardware cloth stage inserted (to facilitate cleaning) at 27°C, 60% relative humidity and 16L/8D photoperiod in FRIUs. Natural diet consisted of excised cotton leafs infested with aphids (*Aphis gossypii* Glover (Hemiptera: Aphididae)). Leaves were then dipped into either a 1.0% L-methionine solution or 0% L-methionine (control) mixed with deionized H_2O. Five adults were used in each replicate for a total n=30 for each treatment. Leaves were replaced every other day from 27 October 2002 to 07 November 2002. Artificial diet was obtained from ENTOMOS and prepared according to their guidelines with the exception of the inclusion of methionine for the 1.0% L-methionine treatment (wt/wt). Diets were replaced every other day from 27 October 2002 to 07 November 2002. Ten adults were used for each replicate for a total n=60 for each treatment. Data was normalized to 0% mortality when the treatments were corrected for control mortality (i.e., when the control mortality was greater than that of the treatment).

Neochetina eichhorniae

Adults of the MWHW were used in this study since the larvae and pupae are buried deep in plant tissue and therefore not likely to come into contact with methionine that could be present in a body of water. Specimens were supplied by Hydromentia, Inc. (Ocala, FL), from areas around South Florida. Weevils were maintained following the procedures outlined by Haag and Boucias (1991), with small petri dishes fitted with moistened filter paper and freshly cut water hyacinth leaves. Water hyacinth plants were collected from Lake Alice on the campus of the University of Florida and maintained in the University of Florida, Department of Entomology and Nematology greenhouse. Treatments consisted of cut leaves dipped in deionized H_2O (control) or solutions containing 0.1% L-methionine, 0.5% L-methionine, 1.0% L-methionine or 1.0% proline.

Prior to weevil exposures, each leaf was inspected for feeding scars or damage and noted to ensure the counts were based on current feeding. Each treatment consisted of 4 replicates with n=5 per replicate (n=20 per treatment and total n=100). Weevils and hyacinth leaves were held in 26.4L x 19.2W x 9.5H (cm) clear plastic boxes with a hardware cloth (to facilitate cleaning) and maintained at 27° C, 60% relative humidity and 16L/8D photoperiod in FRIUs. Fresh leaves were provided every 4 days; exposed leaves were preserved in sealed plastic bags and placed in a refrigerator until scars could be counted. Feeding damage was determined (with the use of an Olympus Tokyo Model 213598 stereo microscope) by the total number of scars present with each counted scar marked with a fine tipped permanent marker (Figure 7-3).

Statistical analyses of the weevil data were performed using Minitab Version 12 (Minitab, Inc.; State College, PA). Feeding scars on control and treatment leafs were

compared with a One-way ANOVA and mean separation was performed using Tukey's Multiple Comparison test (Zar, 1999).

Lysiphlebus testaceipes

To test the effects of methionine on the GBP, cotton plants (*Gossypium sp.;* Family: Malvacae) were grown and maintained at the University of Florida, Department of Entomology and Nematology green and shade houses from 07 October 2002 to 25 November 2002. Aphids (*A. gossypii* Glover) were supplied from other experiments using this organism and kept on plants within a sealed greenhouse to prevent unwanted parasitism. Plants were maintained in the sealed greenhouse, infested with aphids and then placed in the open shadehouse area to encourage parasitation. In total, 20 plants were used for 2 treatments, 1.0% L-methionine and 0% L-methionine (Control) mixed with deionized H_2O. Plants were sprayed weekly (12 October 2002 through 17 November 2002) with approximately 10 ml of solution using a hand-held spray bottle. Counts of parasitized aphids began approximately two weeks after placing plants outside to ensure adequate time for parasitism (Royer *et al.* 2001). Counts were made using a hand lens and counter; "mummies" with exit holes were enumerated and removed. A few parasitized aphids were removed and held in glass vials to ensure correct identification of the parasitoid.

Data Analysis

Data from the parasitoid experiments were analyzed using Minitab Version 12 (Minitab, Inc.; State College, PA). Control and experimental plants were compared against one another with a One-way ANOVA and separation of significant means was performed with Tukey's Multiple Comparison test (Zar, 1999).

Results

Coleomegilla maculata

There was virtually no difference between the control and treatment groups for either the artificial or natural diet tests after correction for control mortality. Mortality was slightly higher for the control groups than the 1.0% L-methionine treatment (Figures 7-1 and 7-2). Further analysis was not necessary because of the identical numbers.

Neochetina eichhorniae

Total mortality for the treatments was less than 20% for all treatments, with the individual treatments having similar results (Figure 7-4). Feeding damage ranged between 2,000 and 4,000 scars per treatment and an average of 10.7 to 16.9 scars per survivor during the course of the experiment (Figure 7-5). No statistical differences were observed between the treatment and control groups

Lysiphlebus testaceipes

In total, 188 and 232 aphid mummies with exit holes were found on treatment and control plants, respectively. Means for each treatment were not statistically different for each collection period or overall based on One-way ANOVA (Figure 7-6) with the only exception being the second and last collection period.

Discussion

In general, L-methionine did not have the same toxic effect on the non-target organisms tested when compared to the pest species exposed to the compound in previous chapters. The pink spotted ladybird beetle adults actually showed the least amount of susceptibility to L-methionine. Survival of the adult beetles was higher in the 1.0% L-methionine treatments than the control for both the artificial and natural diet

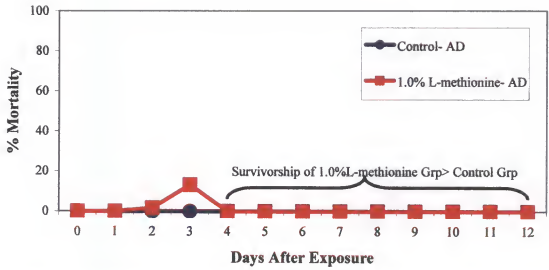

Figure 7-1. Mortality of *Coleomegilla maculata* adults after exposure to L-methionine treated artificial diet. Data corrected for control mortality using Abbott's formula.

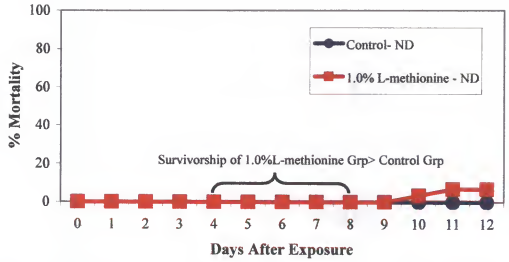

Figure 7-2. Mortality of *Coleomegilla maculata* adults after exposure to L-methionine treated cotton plant leaves infested with aphids. Data corrected for control mortality using Abbott's formula.

Figure 7-3. Feeding scars on water hyacinth (*Eichhornia crassipes*) leaf after exposure to *Neochetina eichhorniae* adults. Black marks represent feeding scars marked with a fine tip marker to aid in counting (other side counted but not shown).

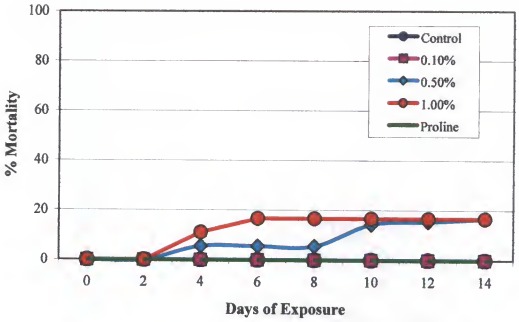

Figure 7-4. Mortality of *Neochetina eichhorniae* on treated water hyacinth leaves. Data corrected for control mortality using Abbott's formula.

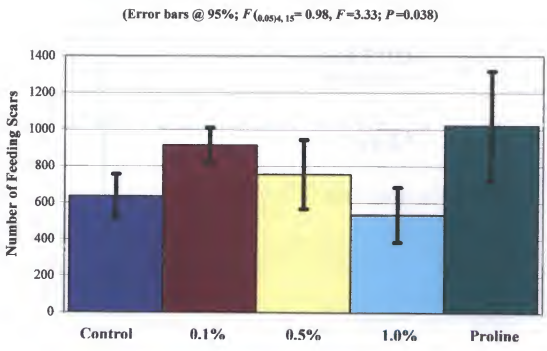

Figure 7-5. Feeding rate of *Neochetina eichhorniae* on water hyacinth leaves treated with L-methionine and Proline. No statistical differences were observed between treatments (Tukey's MST, P=0.038).

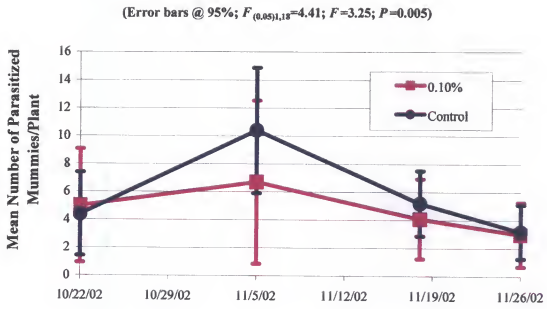

Figure 7-6. *Lysephlebius testiceipes* parasitized aphids on cotton plants treated with L-methionine. Ten plants were used for each treatment and held in the shade house at the University of Florida, Department of Entomology and Nematology from 22 October to 25 November 2002. No statistical differences were observed except for the second and final collection date.

trials. One possible explanation for this observation could be that the excess L-methionine increased the dietary quality of the artificial and natural diets for the PSLB in the treatments. However, because only adults were available, further tests are needed to determine if the larvae, also predaceous on the same pests as the adults, are sensitive to this compound. It should be noted that the midgut properties (i.e., alkalinity) for this species are not well known and may not even have the CAATCH1 proteins present in the midgut.

The mottled water hyacinth weevil also appears not to be adversely affected by exposure to excess amounts of L-methionine despite its herbivorous habit like the THW and CPB. Another weevil within the same family (*Anthonomus grandis* Boheman (Coleoptera: Curculionidae)) is known to have an acidic midgut and the same could apply to the MWHW based on these results (Nation 2001). Therefore, this species and possibly other weevils may not be affected by compounds like L-methionine because of the lack of an alkaline midgut needed for the CAATCH1 protein to operate (Feldman *et al.* 2000; Quick and Stevens 2001). Again, further research is necessary to determine if CAATCH1 proteins are present in this weevil species.

The greenbug parasitoid also was unaffected by exposure to the excess L-methionine found on treated leaves infested with aphids. Dadd and Krieger (1968) found higher methionine requirements for the greenbug *Myzus persicae* Sulzer (Hemiptera: Aphididae) when cysteine is scarce because of its ability to transform excess methionine to much needed sulfur and could possibly explain the parasitoid's tolerance to high methionine concentrations. Because of the life cycle of the GBP, and many other parasitoids, direct contact with compounds such as L-methionine would occur inside the

body of the host, and not through direct contact with the foliage where the compound was applied. There is a possibility for the parasitoid having higher methionine requirements; based on filarial worm infected *Aedes aegypti* (L.) (Diptera; Culicidae) females and the associated drop in methionine levels in the haemolymph (Jaffe and Chrin 1979). This makes alternatives such as L-methionine safe for use around beneficial insects like the greenbug parasitoid.

Overall, the results indicate that the PSLB (*C. maculata*), the MWHW (*N. eichhorniae*) and the GBP, (*L. testaceipes*) were not adversely affected by exposure to L-methionine in excess concentrations in a variety of artificial and natural diets. Survivorship and feeding rates were not statistically different between control and treatment groups for each species. From these data, it can be concluded that L-methionine is safe for use with beneficial insects and could be considered "biorational" in that it showed no adverse effects on non-target species. It also should be stressed that additional testing on other beneficial insects would be, on a case by case basis, necessary to examine the safety and "biorational" qualities of L-methionine.

CHAPTER 8
SUMMARY AND DISCUSSION

The creation and implementation of Integrated Pest Management (IPM) strategies to combat pest species were developed as a response to the economic losses associated with the overuse of chemical control. However, IPM strategies are not widely used because of the lack of alternatives and the ease of use of pesticides. This has resulted in the resistance to pesticides in many insect species, including economic and medical pests. In an effort to provide alternatives to traditional chemical control, biorational methods have been investigated and one such avenue is the use of non-protein amino acids.

Chapter 2 covered the history of the use of non-protein amino acids as a pesticide, and discussed the CAATCH1 system and the safety of L-methionine. Only a handful of these amino acids have been investigated as a means of controlling insect pests but still lack the practicality and cost effectiveness as current chemical control methods. Recent discovery of a new midgut membrane protein, CAATCH1, has revealed a new possibility in insect control. The CAATCH1 system works in alkaline conditions and responds to different amino acids, mainly the reduction in ion flow after exposure to methionine, an essential amino acid required for normal development and metabolism of many species including humans. The use of a compound such as methionine would be an excellent addition to the IPM arsenal because of its relative safety to vertebrates and warrants further study as a pesticide.

Chapters 3,4, and 5 were dedicated to examining the effects of L-methionine, a common analog of methionine, on three different economic and medically important

pests. The tobacco horn worm (THW), Colorado potato beetle (CPB) and the yellow fever mosquito (YFM) were tested and found to be susceptible to concentrations greater than 0.1%. Diets, both natural and artificial, containing this compound resulted in the complete mortality of THW and also in the natural diet for CPB. Development and feeding rates were also affected by the addition of L-methionine to diets for THW and CPB. Survivorship and developmental rates of YFM were also affected by the addition of this amino acid to the larval habitat.

In Chapter 6 it was found that the field application of L-methionine under natural conditions was able to control CPB. It was also determined that L-methionine was compatible with Silwett L-77, a commonly used adjuvant, and showed no detrimental effects on crop yield of eggplant.

Finally, the application of a compound such as L-methionine has to be able to control the pests that it is used against and not have an effect on beneficial organisms that may come into contact with this compound. Chapter 7 detailed the results of tests that involved various beneficial insects from different feeding guilds (herbivore, predator and parasitoid) showed that L-methionine does not appear to pose a threat to nontarget organisms.

One aspect of the use of a compound like L-methionine that is very important is the relative safety. The health hazards related to the contamination of the environment with pesticides are well documented and in the recent years have resulted of the review and removal of several insecticides from commercial and private use. The use of L-methionine as an insecticide would alleviate the dangers associated with other pesticides. The approved use as a nutritional supplement for livestock feed is a testament

to the safety of this compound and residual found on the plant does not pose the same risk to the human population.

It is difficult to understand how a compound such as methionine can be considered essential and deadly within the same organism. To understand this dichotomy, an examination of the role of this compound and how it relates to metabolism, development and reproduction is necessary.

Although the diet of the THW is lacking high concentrations of methionine, the use of hexamerins may account for the levels needed for the biosynthesis of JH. The larvae take in methionine, metabolizing what is needed and storing the rest for later on during metamorphosis. In contrast, the larvae of the diamondback moth (*Plutella xylostella* (Lepidoptera: Plutellidae)), feeding mainly on methionine-rich crucifers, lack hexamerins with high methionine concentrations (Wheeler *et al.* 2000). The levels of methionine encountered in a normal diet are below what the CAATCH1 proteins are capable of processing and may also be affected by the presence of symbiotic bacteria that is responsible for methionine oxidation in some insects (Gasnier-Fauchet and Nardon 1986a; 1986b). It is when the concentration exceeds the handling capacity of the midgut that problems occur. The time it takes to digest material containing natural amounts of methionine could be long enough for the CAATCH1 system to recover from exposure. The difference between the artificial and natural diet LC_{50} for the THW (Figure 3-8) appears to support the idea that bound methionine (*i.e.,* incorporated into the diet and not applied topically) takes longer to cause problems for the organism (if any) versus the relatively quick kill associated with the free methionine present on the leaf surface. The

target ingests the methionine first as it feeds ensuring the overload CAATCH1 system and eventually death.

As for the stored methionine, it is released from the storage proteins as needed to synthesize juvenile hormone and allow for transformation in addition to other functions. The remaining methionine is then used for protein synthesis in the tissues around the ovaries to boost yolk production, as seen in the transfer of methionine from male to female *Drosophila* species (Bownes and Partridge 1987). In the THW, the presence of hexamerins with high methionine content may be an alternative to the male contribution possibly found in its ejaculate. Methionine-rich hexamerins are common in Lepidoptera and have been shown to provide the larvae a source of amino acids during the synthesis of these proteins during the last stage of larval development (Wheeler *et al.* 2000). In addition to the need for methionine for metabolism and reproduction, the release of methionine may also in part account for the decrease in ion transport of the posterior region of the midgut during larval molts and the wandering stage present before pupation. Currently, little is known regarding the mechanisms involved with the decrease of ion transport during these developmental stages (Lee *et al.* 1998). Clearly there appears to be more to the role methionine plays in the development of some insects other than the vague designation of "essential" amino acid.

Insects have evolved to deal with limiting resources, such as methionine, and have successfully found effective strategies like hexamerin storage or alternate pathways to deal with such problems. No attempt to link together all the aspects of the role of methionine in a whole organism or system context. It appears that methionine actually may play a role far more important than that of just an essential amino acid. From the

synthesis of homocysteine to produce methionine to the presence of methionine rich hexamerins and allophorins and protein synthesis, the role of methionine in plant-insect interactions may be larger than originally theorized.

The production of methionine overproducing plants could also be used in future IPM strategies. Preliminary results indicate that genetically modified plants do produce enough methionine to affect the survivorship of caterpillars feeding on the plant (unpublished data). This could be used in crops in which improved nutritional quality is important as well as the insecticidal properties of the additional methionine. However, there appears to be a sublethal level (0.1%) of L-methionine in which THW and CPB can "tolerate" and survive with little mortality (Figures 3-9 and 4-1). Any system that makes use of a crop that can overproduce compounds like L-methionine would have to be able to express levels greater than this level to avoid any resistance/tolerance.

This research has also provided more possibilities for the use of compounds such as L-methionine in the YFM portion of this study. The amino acid Beta-alanine provided similar levels of control, as did the methionine trials (Figure 5-7). Although unexpected (as discussed in Chapter 5), it shows that there are several other systems that can possibly be exploited in controlling some insects.

Further research is necessary to determine if the combination of a compound like methionine and a pesticide already in use would result in the increase in toxicity or the decrease in the concentration of pesticide used. If compatibility between methionine and *Bacillus thuringiensis* does exists, then it is possible that resistance could be broken in a given population. For example, if a population of THW started to show resistance to *Bacillus thuringiensis kurstaki* then methionine could be used to remove both susceptible

and resistant alike because of the difference in mode of action. Once the population was reduced, and the corresponding resistant genotype, *Btk* could be used once more at a lower concentration, closer to that of the susceptible population. This system could also be used for the reduction of *Bt* toxin resistance in the CPB and YFM if the compounds are compatible.

In conclusion, it appears that L-methionine can be used as an insecticide to control insect pests of economic and medical importance. The target site (CAATCH1) is known and found in the midgut/hindgut (presumed) in at least three pest species (tobacco hornworm, Colorado potato beetle and the yellow fever mosquito) and possibly more. The compound (L-methionine) is a safe compound that is already used for livestock feed supplements, has very low mammalian toxicity, and is compatible with insecticide application systems. Non-target organisms were not affected with the application of L-methionine, further supporting its use as a biorational insecticide. With increasing resistance to current insecticides in the study organisms, alternatives such as L-methionine are needed now more than ever to further support of Integrated Pest Management strategies.

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BIOGRAPHICAL SKETCH

Lewis Scotty Long was born in Calhoun, Georgia on August 20, 1971. He graduated from Madisonville High School (Madisonville, Tennessee) in May 1989. On a biology scholarship, Lewis attended Middle Tennessee State University (MTSU), where he earned his BS in May 1994. On graduation, he took a job as an aquatic biologist for Aquatic Resources Center (Franklin, Tennessee). Lewis worked there specializing in taxonomy of mayflies, stoneflies, caddisflies, and freshwater molluscs (snails and mussels). While still employed at Aquatic Resources Center, he started his graduate studies in 1996 at MTSU and continued the work he had started during his undergraduate years. In May of 1999, Lewis graduated with his MS. After receiving his MS, Lewis moved to Florida and entered the PhD program at the University of Florida, Department of Entomology and Nematology. He worked with Dr. Bill Peters (Florida A&M University) on the worldwide taxonomic revision of an understudied group of mayflies. However, Dr. Peters unexpectedly passed away in 2000, and Lewis took this unfortunate event as a chance to broaden his expertise in entomology. In 2000, he took a part-time job with Drs. James Cuda and Bruce Stevens on research that was in the patent process. This was the research that Lewis undertook for his dissertation. Lewis also served as a teaching assistant for the department for classes such as Bugs and People, Life Sciences for Education Majors, Principles of Entomology, and Medical and Veterinary Entomology. He served as primary instructor for Insect Classification and Immature

Insects. Lewis, along with fellow graduate student Jim Dunford, were awarded the Outstanding Teacher Award by the Entomology and Nematology Student Organization of the University of Florida for outstanding teaching accomplishments in the department.

While at the University of Florida, Lewis joined the U.S. Army Reserve as a medical entomologist. He was assigned to the local Medical Detachments, and served there from 2000 to 2004. Originally he had planned on graduating in 2003, but was called to active duty with the 1469th Medical Detachment as a part of Operation Enduring Freedom (OEF). Lewis was the OEF Theater entomologist, and served as the Executive Officer (responsible for the deployment of personnel and equipment to South West Asia). He was stationed at Kandahar Airfield, where he performed his duty and was awarded an Army Commendation Medal for his work in protecting soldiers from health hazards and diseases associated with the area. Lewis returned and continued his work toward graduation.

Lewis was married in August 1992 to Karen Abbott, and is the father of Emilia Irene (1994) and Bryan Scott (1997). Lewis plans on having a career in the military as a medical entomologist, and all look forward to seeing the world and the rest of their future.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

James P. Cuda, Chair
Assistant Professor of Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Bruce R. Stevens, Cochair
Professor Physiology and Functional Genomics

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

George A. Gerencser
Professor of Physiology and Functional Genomics

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

James E. Maruniak
Associate Professor of Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Simon S.J. Yu
Professor of Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Susan E. Webb
Associate Professor of Entomology and Nematology

This dissertation was submitted to the Graduate Faculty of the College of Agricultural and Life Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

May 2004

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